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PATHOLOGY OF THE BLOOD

A TREATISE ON THE GENERAL PRINCIPLES AND SPECIAL APPLICATIONS OF HEMATOLOGY.

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BY

JAMES EWING, A.M., M.D.,

PROFESSOR OF PATHOLOGY IN CORNELL UNIVERSITY MEDICAL COLLEGE, NEW YORK CITY.

SECOND EDITION, REVISED AND ENLARGED.

ILLUSTRATED WITH FORTY-THREE ENGRAVINGS, AND EIGHTEEN COLORED PLATES DRAWN BY THE AUTHOR.

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TO

PROFESSOR T. MITCHELL PRUDDEN, M.D., LL.D.,

IN APPRECIATION OF HIS CONSTANT AID,

FREELY RENDERED TO

THE AUTHOR

DURING A TERM OF SERVICE IN THE LABORATORY OF THE

NEW YORK COLLEGE OF PHYSICIANS AND SURGEONS,

THIS WORK IS

GRATEFULLY DEDICATED.

37493

PREFACE TO SECOND EDITION.

DURING the past two years the contributions to the knowledge of the blood in both its clinical and its pathological aspects have been very numerous, and, in many instances, of fundamental importance. In the present edition of this work the author has endeavored to incorporate the results of these recent studies in such form as will be found available both for the medical practitioner and for the student of hematology. This undertaking has required references in the text to about four hundred new articles or monographs, which have been added to the bibliographical lists. Among the changes thus introduced are considerable additions to the chapter on Technics, the serum test for blood, and the subject of crioscopy. The discussion of the morphology of blood cells has been enlarged and a new plate added. Some noteworthy additions to the knowledge of leukemia have been included in that chapter. The essential features of Ehrlich's theories on immunity have been presented, but the proper limits of the volume do not permit full discussion of this subject. Four new plates and various other figures have been added to the illustrations.

The author acknowledges with thanks numerous valuable suggestions of his critics, and in many instances has been able to follow these suggestions in the revision.

J. E.

PREFACE TO FIRST EDITION.

THE rapid advances in the knowledge of the pathology of the blood and the multitudinous applications of this knowledge in clinical diagnosis which the last decade has witnessed have brought forth several critical treatises on hematology, and still furnish, as the writer believes, abundant reason for the preparation of another such work in English.

Although the clinical bearings of the subject have been partly or fully accessible to English readers in some recent text-books, many later contributions to the pathology of the blood and blood-forming organs have had to be sought elsewhere, often in the original articles. This omission the present work endeavors to supply.

Much of the theoretical discussion in the volume, abstracts of special articles, and reports of cases, have been set in fine print, so as not to encumber the main text, which has been constructed for the student and general reader. The wants of the laboratory worker and special student of hematology have been partly considered in the chapter on Technics and Chemistry, and in the limited references to pathological anatomy.

There are probably always some deficiencies in a treatise closely relating to clinical medicine when that treatise emanates from a pathological laboratory. Yet a comparison of the various extant works on the so-called "clinical" pathology of the blood has convinced the writer that clinical pathology is pathology still, and that a wide experience at the autopsy table and in the microscopic examination of diseased tissues furnishes an absolutely essential standpoint from which to view pathological changes in the blood. The present volume aims therefore to associate changes in the blood as closely as possible with lesions in the viscera, without which combination the former are very often unintelligible.

In the preparation of the work all available sources of information have been freely consulted, and the writer has profited especially by the labors of Ehrlich, Limbeck, Hayem, Lukjanow, Löwit, Grawitz, Stengel, Cabot, and many others. An endeavor has been made to discriminate between authorities, and in all details of important subjects the author has invariably consulted the original sources of information. Works without bibliography have been of little value in this task, and the considerable number of references involved have therefore been included as a feature of the present volume.

J. E.

CONTENTS.

INTRODUCTORY.

ON THE INTERPRETATION OF ANALYSES OF THE BLOOD.

PART I.

GENERAL PHYSIOLOGY AND PATHOLOGY.

CHAPTER I.

TECHNICS.

PAGE
Qualitative tests for blood 23
Volume of red cells and plasma 31
Enumeration of blood cells 35
Hemoglobin, Iron 43
Histological examination of blood 53
Specific gravity 57
Alkalescence 59
Osmotic tension 61
Crioscopy 62
Bacteriological examination 67
Bibliography 68

CHAPTER II.

CHEMISTRY OF THE BLOOD.

Red cells, Leucocytes 71
Serum 75
The Whole Blood. 77
The Blood Ash 78
Urea, Uric Acid, Glucose, Glycogen. 80
Acetonemia, Lipacidemia, Cholemia 83
Specific gravity 84
Osmotic tension 88
Alkalescence, Basic Capacity. 90
Bibliography 93

CHAPTER III.

MORPHOLOGY AND PHYSIOLOGY OF RED CELLS.

Structure and staining reactions 96
Degenerative changes 100
Numbers, Polycythemia, Oligocythemia 104
Bibliography 120

CHAPTER IV.

THE LEUCOCYTES AND LEUCOCYTOSIS.

PAGE
Morphology 123
Numbers and proportions 128
Degenerative changes 130
Leucocytosis, classification 133
Relation to immunity 138
Ehrlich's theories 141
Clinical types of Leucocytosis 146
Experimental Leucocytosis 155
Bibliography 158
Eosinophilia 161
Lymphocytosis 169
Bibliography 173

CHAPTER V.

DEVELOPMENT OF BLOOD CELLS.

Erythrocytes 175
Leucocytes 180
Blood Plates 186
Bibliography 188

PART II.

SPECIAL PATHOLOGY OF THE BLOOD.

CHAPTER VI.

CHLOROSIS.

Etiology 191
Changes in the Blood 193
Regeneration of Blood 197
Varieties of Chlorosis, Pseudochlorosis 198
Bibliography 200

CHAPTER VII.

PROGRESSIVE PERNICIOUS ANEMIA.

Historical 202
Etiology 204
Pathological changes in Viscera 212
Pathogenesis 215
Changes in the Blood 216
Résumé 222
Bibliography 224

CHAPTER VIII.

LEUKEMIA.

Historical 227
Etiology 230

PAGE
Pathological changes in Viscera 234
Pathogenesis 237
Changes in the Blood 239
Diagnosis of Leukemia 249
Bibliography 251

CHAPTER IX.

PSEUDOLEUKEMIA.

Historical 254
Anatomical characters 255
Relation to Leukemia, Pernicious Anemia, Tuberculosis 258
Changes in the Blood 262
Bibliography 266

CHAPTER X.

ANEMIA INFANTUM PSEUDOLEUKEMICA. SPLENECTOMY.

Historical, Etiology 268
Pathological changes in Viscera 269
Changes in the Blood 269
Significance of v. Jaksch's anemia 270
Bibliography 272

Splenectomy.

Effects of Splenectomy in Animals 273
Splenectomy in Man 274
Bibliography 276

PART III.

THE ACUTE INFECTIOUS DISEASES.

INTRODUCTORY SECTION.

THE BLOOD IN FEVER.

Chemical changes, Resistance of red cells 277
Febrile Hydremia 278
Action of Bacteria upon the Blood 279
Bibliography 280

CHAPTER XI.

PNEUMONIA. DIPHTHERIA.

Pneumonia.

Gross changes, Red cells, Leucocytes 282
Bacteriology 289

Diphtheria.

PAGE
Red cells, Leucocytes 290
Effects of Antitoxin 292

CHAPTER XII.

EXANTHEMATA.

Variola 294
Vaccinia, Varicella 297
Scarlatina 298
Measles 300

CHAPTER XIII.

TYPHOID FEVER.

Red cells, Leucocytes 302
Bacteriology 306
Bibliography, on Pneumonia, Diphtheria, Exanthems, and Typhoid Fever . 308

CHAPTER XIV.

WIDAL'S TEST.

The reaction, etc. 311
Bibliography 319

CHAPTER XV.

MISCELLANEOUS INFECTIOUS DISEASES.

Septicemia, Pyemia, Osteomyelitis 321
Appendicitis, Abscess, Erysipelas 325
Acute Rheumatism, Tonsillitis, Whooping-cough 327
Inflammations of Serous Membranes 330
Gonorrhea, Yellow Fever 331
Typhus Fever, Influenza 332
Tetanus, Plague, Malta Fever 333
Actinomycosis, Glanders, Anthrax 335
Bibliography 336

CHAPTER XVI.

SYPHILIS, TUBERCULOSIS, LEPROSY.

Syphilis, Grades of Anemia 338
Effects of Mercury 339
Congenital Syphilis 342

Tuberculosis, Grades of Anemia 343
Tuberculosis of Meninges, Bones and Joints 347
Chemistry, Bacteriology 348

Leprosy 349
Bibliography 350

PART IV.

CONSTITUTIONAL DISEASES.

CHAPTER XVII.

HEMORRHAGIC DISEASES AND DIATHESIS.

PAGE
Purpura Hemorrhagica 353
Hemophilia 356
Scurvy 357
Hemocytolysis 359
Methemoglobinemia 364
Paroxysmal Hemoglobinuria 366
Bibliography 369

CHAPTER XVIII.

MISCELLANEOUS CONSTITUTIONAL DISEASES.

Diabetes 372
Obesity 374
Addison's Disease 375
Osteomalacia 377
Rachitis 378
Myxedema 380
Bibliography 382

CHAPTER XIX.

NERVOUS AND MENTAL DISEASES.

Mania, General Paresis, Epilepsy, etc. 384
Beriberi 386
Chorea, Graves' Disease 386
Bibliography 387

PART V.

GENERAL DISEASES OF VISCERA.

CHAPTER XX.

THE HEMOPOIETIC SYSTEM.

Liver, general considerations, special diseases 389
Esophagus 394
Stomach, general considerations, special diseases 395
Intestines, absorption and depletion, special diseases. 402
Bibliography 405

CHAPTER XXI.

LUNGS, HEART, KIDNEYS.

PAGE
Diseases of Lungs, Asphyxia 407
Diseases of Heart, Malignant Endocarditis 410
Diseases of Kidneys, Uremia 414
Bibliography 418

CHAPTER XXII.

MALIGNANT TUMORS.

Carcinoma 421
Sarcoma 427
Bibliography 428

PART VI.

ANIMAL PARASITES.

CHAPTER XXIII.

MALARIA.

Technics 431
Morphology of Parasites 435
Plurality of species in Estivo-autumnal group 446
Crescents, Flagellate Bodies 450
Development in Mosquito 453
Conjugation 454
Occurrence of Parasites in the Blood 455
Malarial Anemia 459
Leucocytes in Malaria 462
Bibliography 464

CHAPTER XXIV.

RELAPSING FEVER.

Morphology of Spirillum 466
Changes in the Blood 468
Bibliography 469

CHAPTER XXV.

MISCELLANEOUS PARASITIC DISEASES.

Trichina spiralis 470
Distoma hematobium 472
Ascaris lumbricoides, Anguillula stercoralis 473
Ankylostoma duodenale, Bothriocephalus latus 474
Filariasis 478
Trypanosomiasis 480
Bibliography 481

APPENDIX 483

LIST OF PLATES.

PLATE PAGE
I. Normal blood. Triacid stain 97
II. Normal blood. Eosin and methylene blue 124
III. Normal blood. Fixed in alcohol. Stained two hours by the Nocht-Romanowsky method 127
IV. Mild chlorosis. Eosin and methylene blue 194
V. Severe chlorosis. Eosin and methylene blue 198
VI. Progressive pernicious anemia. Eosin and methylene blue . . 218
VII. Secondary pernicious anemia. Eosin and methylene blue . . . 220
VIII. Myelogenous leukemia. Triacid stain 240
IX. Lymphatic leukemia. Eosin and methylene blue 244
X. Degenerating leucocytes in myelogenous leukemia. Triacid stain . 242
XI. Mast-cells. Ehrlich's dahlia stain 248
XII. Glycogenic degeneration of leucocytes. Iodine and potassium iodide in mucilage of acacia 289
XIII. Agglutinated red cells 361
XIV. Developmental cycle of benign tertian parasite 436
XV. Cycle of quartan parasite 443
XVI. Cycles of estivo-autumnal parasite 445
XVII. Flagellating malarial parasites. Cerebral cortex in pernicious malaria 453
XVIII. Conjugating cycle of tertian malarial parasite 455

INTRODUCTORY.

ON THE INTERPRETATION OF ANALYSES OF THE BLOOD.

Plethora. Although the older physicians regarded the existence of a true plethora as an important and well-established fact in pathology, this view, lacking proof, received a serious blow from the work of Ludwig's pupils, v. Lesser and Worm-Muller, and from the authoritative conclusion of Cohnheim. Lesser and Worm-Muller, attempting to produce artificial plethora by transfusion of blood in animals, found that when the red cells were thereby increased 28 per cent. the numbers fell to normal on the following day, and when increased 58 per cent. the blood became normal on the twenty-third day, while the total quantity of blood might be doubled without abnormal symptoms, owing to the rapid return to the normal quantity. Likewise, Hamburger was unable to produce a permanent plethora in horses, by the injection of large quantities (7 litres) of 5 per cent. solution of sodium sulphate, finding that the isotonic power of the blood, thus increased, became normal in one-half to two hours, while nearly all traces of the salt disappeared from the blood in twenty-four hours. Instead of drawing from these data the conclusion that continuous plethora cannot be induced artificially, the unwarranted claim was advanced that a true plethora does not exist (cf. v. Recklinghausen, Lukjanow). Against this conclusion stood the daily observation of clinicians and pathologists, at the bedside and in the dead-house, that there are extreme variations in the quantity of blood in the vessels of different subjects, in divers states of health and disease. Oertel especially strongly maintained, without being able to offer proof, that the volume of blood might be reduced in endocarditis and with good therapeutical effect. Positive data on the question were gathered by Bergmann and Heissler, pupils of Bollinger, and the fact established that there is, in general, a direct ratio between the volume of blood and size of the heart, and the muscular development of the subject, and an indirect ratio with the subject's fat deposits. Though lacking full experimental proof it is now generally accepted that the quantity of blood in the body is variable, may be increased by hygienic measures, and is diminished in many unhygienic and pathological conditions.

Anhydremia, *i. e.*, a reduction in the volume of blood with concentration of solids, must be admitted to result from loss of body fluids, as by sweating and diarrhea, or by diminished ingestion of water.

Czerny exposed cats in a warm, dry chamber for thirty-six hours, finding that they lost 45 per cent. of their weight, the volume of blood was greatly reduced, its viscosity increased, and the red cells rose, in one case, to ten millions per cubic millimetre.

Grawitz found that profuse sweating is followed in the majority of cases by a concentration of the blood, in one instance from specific gravity 1040 to 1051. A few subjects showed a contrary reaction and diminution in gravity (1060 to 1057.5), which Grawitz refers to nervous influences.

Limbeck examined the blood of a case of cirrhosis of the liver with extreme ascites, before and after tapping the abdomen. On the day after the removal of 18 litres of fluid the red cells had risen from 3,280,000 to 5,160,000. Stintzing and Gumprecht have made similar observations before and after the removal of large serous exudates.

Grawitz found the specific gravity of the blood at first decreased by the intravenous injection of concentrated salt solutions (absorption of water), while the administration of the salt by mouth concentrated the blood.

It therefore follows that the ingestion of water and the loss of body fluids always produces a more or less transitory effect on the volume of the blood. There can be no doubt also that the prolonged sweats of phthisis and the severe diarrhea of typhoid fever, dysentery, and cholera lead to a more or less continuous reduction in the volume of the blood and a concentration of blood cells.

A very striking illustration of this principle was frequently encountered by the writer among the soldiers at Camp Wickoff (1898). When these patients, suffering from prolonged malaria with severe anemia, were attacked by typhoid fever or acute dysentery, the ordinary watery character of the expressed blood drop disappeared, and the blood soon became thick and deep red.

Herz claims to have recognized an "acute swelling" of the red cells in febrile conditions, especially in typhoid fever, and v. Limbeck, finding a considerable average increase in the volume of the red cells in eight febrile cases, believes that relative oligoplasmia with increased volume of red cells is of frequent occurrence in high fevers.

The effect of nervous influences in altering the quality of the blood in the whole or a part of the circulation has been demonstrated by numerous studies.

Cohnstein and Zuntz found that section of the cord above the origin of the splanchnic nerves is followed by general dilatation of bloodvessels, and reduction in the proportion of red cells.

Grawitz and Knopfelmacher found, in general, that vasomotor paralysis is followed by local and general increase in the volume of blood, with diminished specific gravity and proportion of red cells, while the opposite effects follow vasomotor constriction of vessels.

The nervous stimulus of cold baths has been found by Leichtenstern, Wick, Knopfelmacher, Winternitz, Thayer, and Grawitz to be followed by contraction of vessels and increase in the proportion of red and white cells, while the hot pack and the inhalation of amyl nitrite have an opposite effect.

To a similar origin must be attributed the increased gravity of the blood observed by Grawitz after the injection of tuberculin, and cultures of the bacillus of cholera and of diphtheria, and the opposite effect produced by *streptococcus* and *staphylococcus pyogenes* and *B. anthracis.*

The influence of **psychical emotions** on the character of the blood in different regions of the body has been emphasized by Lloyd Jones, and is seen especially in the study of the blood in neurotic women. Jacobi, examining the blood of an hysterical woman, found on November 12th 3,892,000 red cells; on December 11th 8,084,000 red, 102,200 white cells; on December 16th 3,393,000 red, 22,000 white. Grawitz refers to the remarkable increase seen in the specific gravity and proportion of cells in the blood of rabbits, in experiments conducted without anesthesia. The remarkable variations in the blood taken from the ears of rabbits, depending on the temperature and blood-content of the member, are a matter of common observation.

In the *mechanism* by which these changes are brought about there appear to be many factors. Heidenhain, Lowit, Gärtner and Romer, find that many substances injected into the blood cause an increased flow of lymph. (Extract of cancerous tumors, peptone, tuberculin, toxins of *B. pyocyaneus* and *pneumococcus*, hemi-albumose, nuclein, uric acid, etc.) Heidenhain and Hamburger believe that some substances excite a secretory action of the capillary endothelium, whereby the fluids of the blood are diminished.

Grawitz explains the concentration of the blood under the influence of cold by the escape of fluids into the tissues, but the experiment of Cohnstein and Zuntz offers another explanation, since they found under the microscope that capillaries might be so reduced in calibre by irritation of vasoconstrictor nerves that no red cells, but only plasma, could pass in them. It then appears that many red cells may be caught in the contracted capillaries while the plasma passes on into the veins. Grawitz concluded from many comparative tests that the capillary blood is, in all ordinary conditions, richer in cells than that of the veins; but Becker found no such differences in the cells of veins and capillaries, and thinks that the polycythemia of cold is referable both to the escape of fluids from the blood and to the sifting of cells by the contracted capillaries. It does not appear to have been proven that the vascular dilatation produced by heat is followed by the return of tissue fluids into the capillaries. It appears quite as likely that an irregular distribution of cells and plasma is to a larger extent responsible for such local variations in the composition of the blood (cf. Winternitz).

The law that increased blood pressure leads to transudation, authoritatively stated by Ludwig and Landois, appears to have a less important application here than in more general and more prolonged processes. It is not likely that a cold bath can be followed by much exudation of serum into the tissues.

Massage and electricity have been shown, by Mitchell and Cheron, to cause an immediate increase in the number of red cells in the blood of a part, an effect which may be explained according to the above data.

Ekgren found that ten minutes of active massage of body or abdomen increased the peripheral leucocytes, chiefly the polynuclears, from 1000 to 3000, once 7000. The increase was not marked ten minutes after the massage. Oliver was able to lower the red cells of the finger 7 per cent. by elevating the arm, while elevation of the leg for fifteen minutes lowered the red cells of the toe 14 per cent., and raised them in the finger 3 per cent. Exercise and faradism caused an increase of 5 to 8 per cent. in the peripheral blood. A Turkish bath caused an increase of 15 per cent.

The importance of the osmotic relations of the blood in controlling the volume of red cells and plasma is indicated from the discussion in Chapter II. In hydremia and anhydremia Hamburger has shown that the isotonic relations of the blood are maintained by a rapid interchange of salts and albumins between the cells and plasma, with frequent minor changes in their relative volume. From Limbeck's analyses of venous and arterial blood it appears, also, that the imbibition of CO_2 causes swelling of red cells with absorption of water and salts and with relative diminution in the volume of plasma.

The foregoing considerations are dwelt upon not only because of their theoretical interest, but because they deal with fundamental facts without the knowledge of which it is impossible to properly perform or sensibly interpret the results of an examination of the blood.

To summarize the discussion, it has been shown that there are wide variations in the quantity and quality of the blood referable to diverse conditions other than disease.

1. There are considerable physiological variations in the volume and composition of the blood, according to the constitution of the individual (plethora), and the degree of muscular development. Here may be classed the variations between the sexes and between different periods of life. Such variations are permanent but usually not of extreme grade.

2. There is a great variety of physiological conditions producing marked but transitory changes in the blood, such as active digestion, muscular exertion, the ingestion of fluids, profuse perspiration, temporary cyanosis, etc.

3. The nervous system has a very striking temporary influence on the quality of the blood in local or general areas, acting through the cerebral (psychical) or medullary centres, or through local vasomotor nerves.

4. Various local influences may greatly change the quality of the blood specimen, as seen in the local and transient effects of cold, heat, massage, and electricity.

5. Many therapeutical procedures may temporarily alter the blood, as the aspiration of fluids, administration of diaphoretics, purges, vasodilators (amyl nitrite), vasoconstrictors, etc.

6. Various pathological conditions may partly or completely obscure the real status of the blood, as the sweats of phthisis; the diarrhea of typhoid fever, dysentery, and cholera; general cyanosis or local stasis; the increased arterial tension of uremia; the polyuria of diabetes and nephritis; antemortem cardiac failure, etc.

Having regard to the possible action of any of the above influences, one may avoid many of the local disturbances by observing special care in the manner of expressing the blood specimen.

The blood should be expressed by very slight pressure, exerted at a distance, from a liberal puncture of the finger-tip or ear-lobe. The circulation in the part should be as nearly normal as possible and should be uniform. A cold, bloodless tissue is not suitable for furnishing a blood specimen, and if artificial means are taken to correct the condition a sufficient period must elapse to allow the accelerated circulation to subside. Blood should be taken not less than four hours after a hearty meal, and when comparative tests are made the specimens should be taken at the same hour each day.

The examination having been performed, *its results are to be interpreted only in the light of the fullest possible clinical information.* There can be no doubt, as Grawitz has pointed out, that the numerous contradictory results of hematological studies are largely referable to hasty conclusions drawn from figures without regard to the condition of the patient or the stage of the disease under consideration. Likewise, hematological diagnosis has fallen into much discredit from the tendency to offer opinions from the isolated findings of the blood test.

PART I.

GENERAL PHYSIOLOGY AND PATHOLOGY.

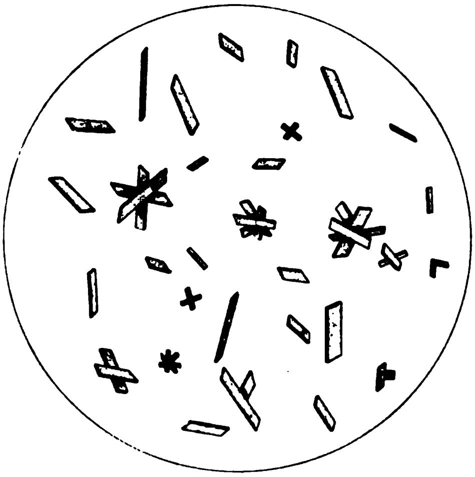
CHAPTER I.

TECHNICS.

QUALITATIVE TESTS FOR BLOOD.

Demonstration of Red Cells. By far the most delicate for blood is the demonstration of red cells under the microscope, but as the cells are not always preserved in demonstrable form one must usually resort to tests for hemoglobin and its derivatives. To demonstrate red cells in *dry blood* stains one should soften the scrapings in some agent which will not lake the corpuscles. Many of the fluids recommended for this purpose are active laking agents for dried red cells—*e. g.*, 0.8 per cent. salt; glycerin, 50 per cent.; sodium sulphate, 75 per cent. A strong alkaline solution, as KOH 30 per cent., is effective in separating the corpuscles and does not dissolve them.

FIG. 1.



Hemin crystals. (Rieder.)

The Guaiacum Test.

To a watery solution of the suspected substance are added a few drops of tincture of guaiac, producing a milky precipitate. A few drops of hydrogen peroxide added will, in the presence of blood pigment, produce a distinct blue color.

The tincture of guaiac should be freshly made, and diluted to the color of pale sherry wine. The guaiac must be added before the peroxide, and the blue color must be developed immediately. Many substances turn tincture of guaiac blue, without the presence of peroxide. This test is very delicate, demonstrating one part of fresh blood in several thousands of water. It is most effective with fresh specimens; but after two or three years some specimens fail to react satisfactorily. Brandenburg finds that leucocytes of myelogenous origin, but not the lymphocytes, give this same reaction. The guaiac test demonstrates the presence of peroxidases, which exist in most animal and vegetable tissues.

The Hemin Test (Teichmann's).

A drop of blood or portion of suspected detritus is spread out on a glass slide, mixed with one drop of salt solution and dried at a low temperature. A cover-glass is then laid over the specimen, filled beneath with glacial acetic acid, and the specimen evaporated with higher heat, but without boiling. When the fluids have entirely evaporated the specimen may be mounted in distilled water and examined microscopically for the characteristic crystals of hemin (Teichmann's crystals).

The hemin test, if successful, is absolutely reliable, but often fails, in unskilled hands, from (1) alteration of albumins by excessive heat, preventing the formation of crystals; (2) excess of salt solution; (3) violent boiling which drives off free HCl, thus preventing the formation of hemin crystals (hydrochloride of hematin).

Hemin crystals often fail to form from old blood stains in which the pigment has become comparatively insoluble from the formation of hematin. In such cases a successful result may sometimes be secured by allowing the specimen prepared in the usual way with excess of dilute acetic or hydrochloric acid to stand overnight (Richter).

When the stain is very faint or diffuse, or washed, the blood pigment may be concentrated, as recommended by Wood, by dissolving the material in weak alkaline solution, precipitating the pigment by boiling after the addition of a few drops of saturated solution of sodium tungstate strongly acidified by acetic acid, collecting the brownish precipitate, and performing the usual hemin test upon it.

MEDICO-LEGAL SERUM DIAGNOSIS OF BLOOD.

One fruitful result of the recent trend of study of the mechanism of immunity has been the discovery of a delicate and reliable method of distinguishing human blood from that of lower animals. The first step in the elaboration of this method of serum diagnosis consisted in the discovery by Tschistowitsch that the serum of an animal treated by injections of defibrinated blood or of serum from another species of animal develops the power of agglutinating and dissolving the red cells or of causing a precipitate in the serum of the second animal.

This effect was found to be limited to the species of animal whose blood was used in the injections, while on the blood of other animals the serum remained inert. It soon became apparent that the serum of an animal could be made to develop specific powers in relation to various animal tissues and albumins and even to vegetable albumins, and in 1900 Wasserman pointed out that the biological method was probably capable of separating between all albumins of whatever source.

Almost simultaneously Deutsch, Uhlenhuth, Wasserman and Schutze, and Stern applied the method to the identification of blood for medico-legal purposes, and showed that it was possible thereby to determine the origin of old blood stains.

Deutsch immunized rabbits by defibrinated human blood and produced specific agglutination and solution of the red cells, finding that the test could be applied to minute quantities of blood cells prepared in the hanging drop. His method is of value in dealing with small amounts of blood in which the red cells are still intact, and, as will be seen, gives reliable information regarding the nature of more minute stains than can be treated by the method of precipitation of albumins. The other observers employed defibrinated blood and secured sera which caused precipitates in solutions of stains.

Method of Preparation of the Serum. The rabbit is the animal usually employed, and three or four large healthy ones should be selected. They should be housed under the best hygienic conditions. The writer has had very satisfactory results with chickens, and there are theoretical reasons for preferring this animal to the rabbit. Defibrinated blood squeezed from the fresh placenta or drained from the umbilical cord should be used for the injections, although less potent sera have been obtained by the use of albuminous urine (Stern, Leclainche and Vallée) or of pleuritic exudate (Dinkelspiel, Dieudonne), or of blood from the cadaver (Modica, Ziemke). The placental blood may be poured into 100 c.c. Erlenmeyer flasks containing broken glass or beads, and defibrination accomplished by shaking the flasks for a few minutes. Or the blood may be allowed to coagulate and the serum used which separates after forty-eight hours. The former method is preferable, as it yields a more hemolytic antiserum. From six to eight injections of 5 to 10 c.c. each, at intervals of three to five days, are required to develop a serum of sufficient potency for practical use.

The intraperitoneal is the preferable route, and the animals usually tolerate the injections well. If they lose weight, treatment should be suspended for a week. Strict asepsis in the handling of the blood and in the injections is, of course, essential.

The serum may be tested from time to time by drawing a little blood from the ear vein, and after a sufficient course of treatment the animal may be exsanguinated by exposing the carotid and drawing the blood through a canula; but it is well not to sacrifice the animal, but to bleed from the marginal ear vein, as a very little subsequent treatment serves to restore the potency of the serum in an animal which has once been bled.

The drawn blood should be allowed to coagulate, and the serum drawn off as clear as possible, centrifuged, if necessary, sealed in glass tubes, and kept on ice. Preservation of the active agent may be secured by adding a few drops of chloroform, or by evaporating the serum on glass plates, the dry residue being dissolved in salt solution when needed (Modica and Nobell).

In testing a suspected stain the material should be dissolved in a very little 0.6 per cent. solution of NaCl (c. p.), or in 0.1 per cent. NaOH, as preferred by Ziemke, or, if the stain is old and fails to dissolve readily, in concentrated potassium cyanide with subsequent neutralization with tartaric acid (Ziemke).

The solution, if turbid, may usually be cleared by the centrifuge or by filtration through Schleicher's filter paper (blue ribbon), or through an asbestos filter. Small test tubes—diameter 0.5 cm.—are required in the work.

To the filtrate should be added the active serum *in proportion not greater than 1:50.* In less dilutions spurious reactions may occur.

The writer finds it advisable to prepare several controls in each test, one of known human blood, and one of the salt or other solution used in dissolving the blood stain, one of blood from another animal, one of human blood to which normal rabbit serum is added, and one of the dissolved stain which shall remain untreated. Without such controls a short experience with the method will convince one that errors will not infrequently arise from unknown factors. All of these specimens should be placed in the thermostat.

The reaction is positive when a distinct turbidity appears instantly or within a few minutes, and when a flocculent precipitate forms within three hours. Less definite reactions, such as uniform turbidity, cannot at present be accepted (Ziemke), although sera of moderate potency give only turbidity at first and require more than three hours for precipitation. It is, therefore, important to secure a serum of high potency, while, on the other hand, the very powerful sera are more apt to cause turbidity in heterologous bloods (Strube, Lenossier and Lemoine). On the other hand, the more powerful sera can be safely diluted beyond the point where reactions in heterologous blood can occur.

The reaction does not fail with very old specimens of blood, although it becomes less distinct the older the specimen. Ziemke obtained a cloudiness in three hours from a blood stain twenty-five years old; from blood mixed with earth three years old; from decomposed blood; and from human blood highly diluted with six other kinds of blood. A solution of iron-rust gave no reaction. Uhlenhuth secured positive results with blood of the three months' decomposed cadaver, and with soapsuds containing washed blood. The reaction fails when dry blood has been heated one hour at 130° C. (Ferrai). Solutions of blood stains on leather may precipitate spontaneously, probably from the presence of tannin.

The delicacy of the test is very great, a reaction having been obtained in solutions of blood too weak to give the absorption spectra, and in solutions of 1 of serum to 50,000 of blood (Stern).

Limitations of the Test. The reaction is not, in one sense, specific, and there are only quantitative differences in the results obtained by adding humanized sera to various types of blood. However, these quantitative differences are very great, and in a large series of animals Stern, Wasserman and Schutze, and Nuttall, found none to react to humanized sera in any degree comparable to human blood except that of the monkey. There are several reports in the literature showing that special sera added in slight dilution (1 : 5, 1 : 20) cause reactions in the blood of many animals not very closely related, but it has been fully shown that if the test serum is diluted up to 1 : 50 or 1 : 100 and added to blood diluted 1 : 20 or 1 : 100, the results become absolutely specific. It is necessary, therefore, to attend carefully both to the dilution of the serum to be tested and to that of the active serum used in the tests. Kister and Wolff, and Strauss and the writer have found that most reactions in heterologous bloods may be avoided by diluting the test serum 1 : 50. Indeed, most of them fail to appear when the serum is diluted 1 : 30.

The writer has compared the reactions of human and monkey blood (rhoesus) treated by several potent humanized rabbit sera, and finds that the reaction fails in this monkey blood when the test serum is diluted 1 : 50. The strength of the solution of the blood to be tested, if not greater than 1 : 1000, made comparatively little difference.

The method is not capable of distinguishing the chemical variety of albumin, as Rostocki showed that rabbit serum potent against horse globulin precipitated also serum albumin of the horse.

A more practical difficulty in identifying blood is found in the fact that the active serum precipitates albumins from exudates, urinary and pleuritic, etc., and from sputum. Strauss and the writer secured good precipitates from albuminous diarrheal stools not containing blood. It must, therefore, first be determined by the hemin test that the stain is blood. Contamination by pus, feces, sputum, or urine can usually be detected by appropriate tests.

Sources of Error. 1. Turbidity or precipitate may occur in any blood from too slight dilution of serum.

2. Potent sera considerably diluted (1 : 20) cause turbidity in solutions of blood from closely related animals.

3. The chemicals used may be impure and cause precipitates.

4. Chloroform does not inhibit all bacterial growth, and precipitates occurring after three hours may be referable to this cause.

5. The reaction of the solution may be too acid or too alkaline, and the reaction fails or it occurs in heterologous blood (Ziemke, Rostocki). The reaction should be neutral or slightly alkaline (Lenossier, Lemoine), or slightly acid (Rostocki).

6. The blood may be mixed with chemicals which cause precipitates, or human albuminous or mucous exudates may be present in the solution and react as blood albumins (cf. Graham-Smith and Sanger).

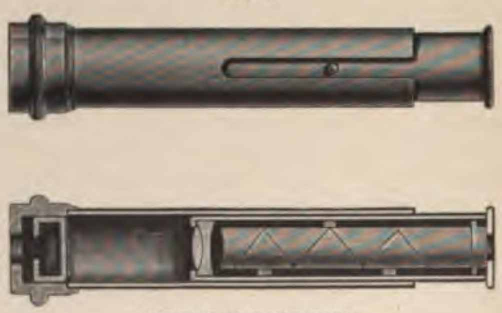
From the foregoing discussion it is evident that full medico-legal requirements for the positive identification of blood stains may be met, under some conditions, by the biological test. These conditions are the positive proof by the hemin test that the material is blood and the occurrence of a flocculent precipitate appearing within one to three hours in the suspected specimen and in no other of the several controls when a potent serum is added in proportion of 1:50 of blood.

While these conditions can usually be secured when dealing with fresh blood in considerable quantities, in the writer's opinion and experience the material submitted for medico-legal examination is more apt to be old, scanty, and impure, and the difficulty of securing a fully satisfactory test by this method is thereby greatly increased. With such material one has often to be content with faint turbidities instead of flocculent precipitates, and in such cases it would appear, as Stoenesco maintains, that a guarded opinion be given and the claim made only that the specimen is probably human blood. It should be added that an absolutely faultless technique is required, and that this can be obtained only after considerable experience.

SPECTROSCOPIC EXAMINATION OF BLOOD.

In all cases where a sufficient quantity of dissolved blood can be obtained for examination the spectroscopic test is the best means of determining not only the presence of blood pigment but also its particular form.

FIG. 2.



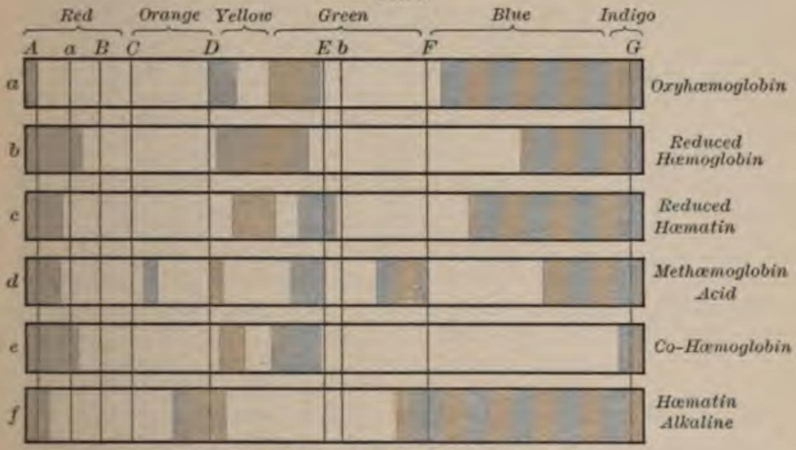
Browning's spectroscope.

Of fresh blood a 1 per cent. solution yields very distinct absorption bands. Recently clotted blood dissolves readily in water. Old clots may usually be dissolved by maceration in acetic acid, after which the spectrum of acid hematin is obtained. Clots that have been exposed to heat must be macerated in ammonia, when the spectrum of reduced or of alkaline hematin will result.

For ordinary clinical work Browning's spectroscope is very satisfactory, but absorption bands are more accurately located in larger instruments. The small instrument should be supported in a convenient holder in strong daylight or gaslight. By means of a collar the width of the aperture may be varied according to the strength of the light and opacity of the fluid. By means of the sliding tube Fraunhofer's lines are brought into accurate focus. The fluids should be examined in small glass vials with flat sides.

When dealing with fresh blood and unaltered Hb the spectrum is that of *oxyhemoglobin,* which shows two absorption bands between *D* and *E,* one rather thin and sharp near the orange, and the other broader near the green. The indigo and most of the blue is absorbed. In strong solutions the two bands of oxyhemoglobin may be united.

FIG. 3.



Spectra of blood pigments. (After Limbeck.)

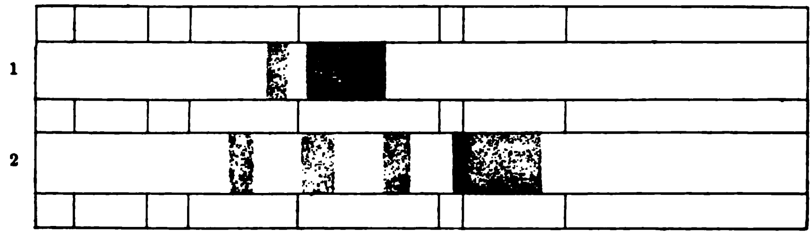
If to the solution of oxyhemoglobin is added a little reducing substance, such as ammonium sulphide, the color of the fluid becomes darker and the spectrum changes, becoming that of *reduced hemoglobin,* giving one broad absorption band between *D* and *E.*

The transformation of the spectrum of oxyhemoglobin to that of reduced Hb, by reducing agents, is the positive indication of the presence of blood. Cochineal and ammoniated carmine give spectra very similar to that of oxyhemoglobin, but on the addition of boric acid the spectra of these substances are displaced into the blue, while that of blood remains unaffected. The spectra of various other vegetable dyes simulate that of blood, but these are bleached by sodium bisulphite.

Hematin is produced by the addition of acids or strong alkalies to reduced Hb. In acid solution its spectrum is similar to that of acid methemoglobin. In alkaline solution it gives a single rather broad band at *D.*

The end-product of the alteration of hemoglobin found in old blood stains is iron-free hematoporphyrin, which must be identified by the spectroscope. Kratter and Hammerl claimed to have obtained the spectrum of this pigment from specimens of incinerated blood. To prepare a solution of hematoporphyrin the suspected stain should be dissolved in concentrated sulphuric acid, which should yield a reddish violet fluid in one-half hour. The spectrum of hematoporphyrin resembles that of oxyhemoglobin, but the bands are displaced further to the left, especially the small band to the left of *D*. In alkaline solutions the spectrum resembles that of acid hematin (Fig. 4).

FIG. 4.



Spectra of hematoporphyrin. (1) in acid solution, (2) in alkaline solution.

Clinically, the most important alteration of oxyhemoglobin is that into *methemoglobin*, sometimes detected by the chocolate color of the blood. In acid or neutral solution it gives *four absorption bands*, one quite sharp, between *C* and *D*; a second, faint, in the yellow, immediately to the right of *D*; a third, broad, rather distinct, between the yellow and green, and just to the left of *E*; a fourth, broad, to the left of *F*, sometimes merged with a complete absorption of the blue end of the spectrum.

The demonstration of *carbonic-oxide-Hb* is of great clinical importance in cases of gas poisoning. The blood drop has a rosy red tinge, seen alike in both venous and arterial blood. In ½ per cent. dilution a spectrum is obtained which resembles that of oxyhemoglobin, but the bands are broader, and the *D* band is displaced slightly to the right. On the addition of ammonium sulphide the spectrum of oxyhemoglobin is replaced by that of reduced Hb, while Co-Hb remains unaltered. In applying this test clinically, since considerable oxyhemoglobin remains in the blood in most cases, the results of spectral analysis are not always clear, and corroborative tests are required.

1. Warm the specimen with equal parts of 10 per cent. NaOH; normal blood becomes dark brownish green, the other becomes cloudy, then clear red, and red flakes gather on the surface (Hoppe-Seyler).

2. To a 2 per cent. solution of blood add a few drops of orange-colored ammonium sulphide containing an excess of sulphur, and faintly acidify with a few drops of dilute acetic acid, *carefully* shaking. Carbonic oxide blood then shows a beautiful rose-red color,

with a flocculent precipitate, while normal blood becomes greenish or reddish gray. The test may be performed in a porcelain dish, adding a drop of blood to the mixed reagents (Katayama).

Kunkel and Welzel employ a solution of zinc chloride, or very dilute solution of platinum chloride, which color carbonic oxide blood bright red, normal blood black.

Rubner recommends that the suspected blood be diluted four to five times with acetate of lead, when normal blood becomes chocolate, carbonic oxide blood red.

Watery neutral solutions of Co-Hb, boiled, yield a clear red coagulum, while oxyhemoglobin becomes grayish brown (Hoppe-Seyler).

Estimation of Total Quantity of Blood.

The limits of error in the methods of estimating the total quantity of blood, suggested by Valentin, Vierordt, Buntzen, and Thibault, probably exceed the physiological and pathological variations in the bulk of this tissue, and are, therefore, not available for clinical purposes.

Haldane and Smith have determined the total quantity of blood in fourteen healthy subjects by a method based on the capacity of the blood to absorb CO_2. In these subjects the total bulk of blood varied between 3.34 per cent. and 6.27 per cent. (average 4.88 per cent.) of the body weight (one-thirtieth to one-sixteenth). The lowest percentage was obtained in a very fat subject. Most physiologists hold that the total blood in healthy animals is about one-thirteenth of the body weight.

ESTIMATION OF THE VOLUME OF RED CELLS AND PLASMA.

The Hematocrit. The idea that the centrifuge might give valuable clinical information concerning the volume of red cells was original with Blix, while his instrument and method have been improved principally by Hedin, Gärtner, and Daland. Although hand centrifuges have been used and recommended, the best results are obtained only with the electric centrifuge, which is at present in the market at a reasonable figure.

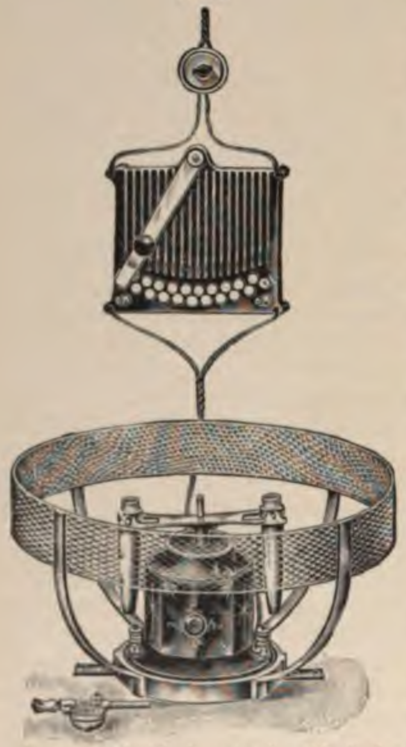
The improved electric centrifuge consists of an iron-clad motor carrying a steel shaft and horizontal armature for urine tubes, which may be replaced by the hematocrit. A "speed indicator" may also be attached, which strikes a bell with every 100 revolutions. The instrument is practically noiseless even with a very high speed. A *rheostat* is used to control the current and speed. A speed of 8000 to 10,000 revolutions may be obtained by a small battery or from the street current. The hand centrifuge (Fig. 6) may be employed when it is not convenient to use electricity.

The hematocrit attachment consists of two capillary tubes, graduated in 100 degrees, which are held in the armature by springs.

Procedure. With walking patients *fresh blood* may be used. The capillary tubes may be filled automatically by holding the tube horizontally, or with one end slightly depressed, and touching it to the rather large drop of blood required. The tube is then immediately inserted in the armature, as in Fig. 6, the opposite tube having previously been filled with water, and the revolutions are begun before the blood coagulates.

When the patient is at a distance the blood must be diluted, preferably in 2.5 per cent. solution potassium bichromate, as recommended by Daland. The blood should be diluted with equal parts of this solution, which may be accomplished in the red-cell or white-cell pipette of the hemocytometer. With the red-cell pipette the capillary tube is filled with blood to the mark 1, then a small air bubble is drawn in, followed by another tube-length of blood. Three or four tube-lengths of blood should be secured in this way and immediately mixed with an equal number of tube-lengths of diluent. With the white-cell pipette a single measure of blood and diluent is sufficient. The blood and diluent should then be mixed by gentle shaking, taking care not to enclose air bubbles. When diluted blood is used both tubes of the hematocrit should be filled with blood, which may be done by allowing the drops to flow in from the point of the pipette.

FIG. 5.



Improved electric hematocrit, with fender, rheostat, and speed indicator. The hematocrit attachment replaces the urine tubes seen in the revolving armature.

The tubes are now revolved at a speed of eight to ten thousand revolutions per minute, for three minutes, after which the volume of the red cells has been found unalterable.

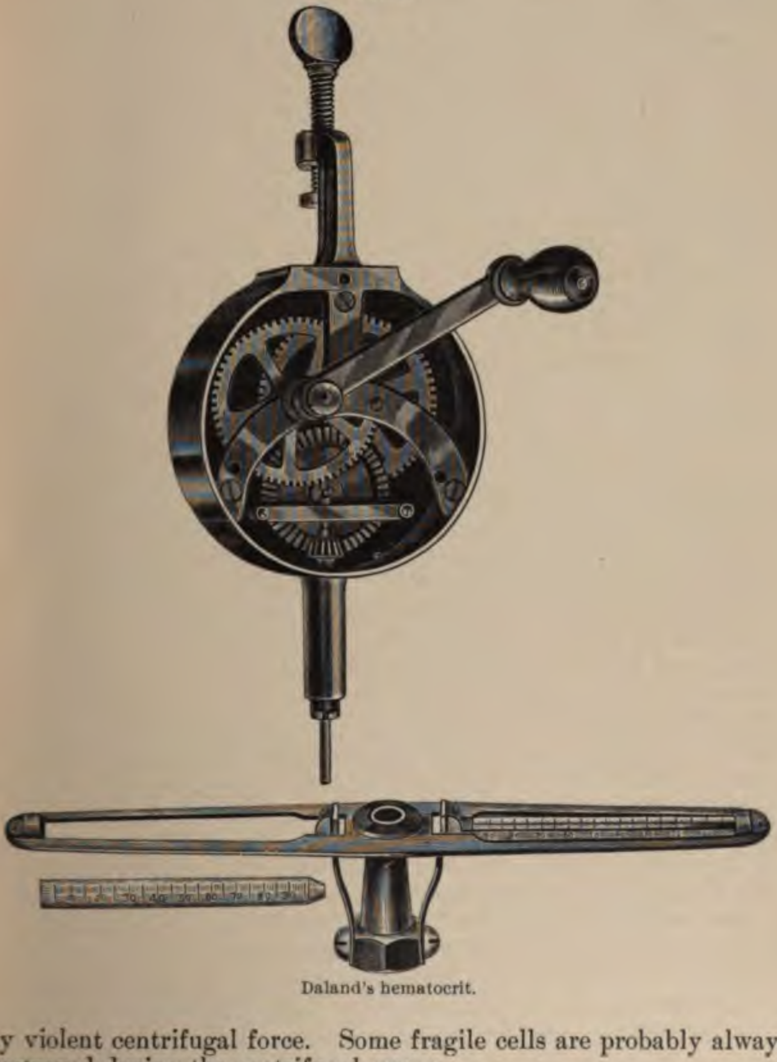
The question now arises how many red cells are contained in one degree of the scale. Daland, working extensively with diluted blood, places the number at 99,390, practically 100,000.

Cabot, in a series of 40 comparative tests, using undiluted blood in the hematocrit, found variations between 105,000 and 123,000, with an average of 112,000.

Further observations on this point are required, but at present the above figures should be used for diluted and fresh blood respectively, multiplying the result by two when diluted blood has been used.

Limitations of the Hematocrit. Since the centrifuge does not necessarily require the use of diluting fluids, a serious cause of error in the method may be removed by the use of fresh blood. It must be admitted, however, that the original volume of the red cells cannot always be exactly determined by this method, as in pathological conditions the compressibility of the corpuscles is not always uniform. Moreover, in altered conditions of the plasma, it is uncertain how much change can be wrought in the natural volume of red cells by violent centrifugal force. Some fragile cells are probably always destroyed during the centrifugal process.

FIG. 6.



Daland's hematocrit.

When diluting fluids are used the error is doubtless increased by alterations in the density and composition of the plasma and in the volume of the red cells caused by the action of the fluid. Nevertheless Daland's claim must be admitted that the volume of the red cells, except in leukemia, is determined by this method with accuracy sufficient for clinical purposes. The value of such information is, of course, quite evident.

The further claim that the hematocrit may give more accurate estimates of the *number* of red cells than does the hematocytometer has not been confirmed. The volume of the red cells differs so markedly in both the chlorotic and the pernicious anemias that one cannot seriously consider the project of replacing the hematocytometer by the hematocrit. Only in the moderate secondary anemias, with little change in the size and Hb-content of the cells, can the volume of the red corpuscles yield reliable indications of their number. In cases of leukemia and of extreme leucocytosis so many leucocytes are entangled with the red cells that even the volume of the red cells is not accurately told, much less their number. Each of these instruments has its proper field to which it should be restricted, and as the hematocrit is not overexact in its immediate object, it is unscientific to introduce a second source of error, as is done in attempting to compute the number of red cells from their volume.

It may be added that the value of the hematocrit in estimating the character and severity of an anemia has not yet been as fully recognized by clinicians as it deserves, possibly because more attention has been paid to the number of red cells than to their functional capacity.

The reliability of the centrifuge in determining even the volume of the red cells has been denied principally by the brothers Bleibtreu, and by Bleibtreu and Wendelstadt. These observers devised another method of determining the volume of the red cells, which they claim gives more trustworthy results than are obtained by the hematocrit. They employed 0.6 per cent. salt solution to prevent coagulation, and allowed the blood to settle slowly. The nitrogen-content of the supernatant plasma was then determined by Kjeldahl's method, and from tables which these observers constructed the volume of the plasma, and hence that of the red cells, could be determined from the quantity of N obtained.

While the results obtained with the hematocrit by several observers indicate that the normal volume of red cells varies between 40 and 66 per cent., Bleibtreu's method gave normal variations in cadaveric blood between 25.15 and 55.8 per cent. (Bleibtreu, Pfeiffer). v. Limbeck obtained very low volumes with Bleibtreu's method (24 to 28 per cent.), which he refers to the use of highly oxidized blood, in which he believes the red cells are reduced in volume. The lengthy discussion which has prevailed regarding the above points indicates that the volume of the red cells is subject to a considerable variety of changes, the origin and significance of which are little understood. It has been shown that in order to prevent N from leaving the red cells during sedimentation, the exact isotonic tension of the plasma must be determined in each instance, and a corresponding solution of salt used. The isotonic tension of plasma is rarely so low as 0.6 per cent. NaCl.

Moreover, supposing that the red cells remain intact during sedimentation, the pathological variations in the N-content of the plasma, depending on several variable nitrogenous bodies, are too frequent and marked to permit of any fixed formula to give the volume of the serum from its content of nitrogen. (Bleibtreu's method has been sharply criticised by Hamburger, Eyckman, Hedin, Biernacki, and others.)

The following table of results obtained by Biernacki well illustrates the unreliability of comparisons between results obtained by different procedures and the general inaccuracy of all indirect methods of estimating the number of red cells or percentage of Hb from the volume of the cells and the specific gravity :

Case.	Content of water.	Red cells.		Hb.
		Number.	Volume %.	
1. Normal	77.18 %	5.037 mil.	56.3	105 %
2. Normal	77.50	5.487	53.6	100
3. Cancer of esophagus	79.58	5.175	52.7	80
4. Rheumatism	79.02	3.902	49.1	85
5. Chlorosis	80.99	4.958	50.0	70
6. Phthisis	82.37	4.672	40.9	60
7. Nephritis	82.73	4.800	40.0	70
8. Tabes	83.09	4.512	47.6	65
9. Chlorosis	83.04	4.250	35.4	55
10. Phthisis	84.59	1.975	30.9	50
11. Ulcer of stomach	85.43	3.825	37.1	45
12. Chlorosis	89.36	2.456	20.0	25
13. Nephritis	89.46	1.184	13.6	20

Grawitz determines the volume of red cells in blood drawn in considerable quantities by venesection. The specific gravities of the whole blood (D_1), of the centrifuged serum (D_2), and of the sedimented red cells (D_3), are first determined, from which the volume percentage of red cells (x) may be computed by the following formula :

$$x = 100\frac{(D_3 - D_1)}{(D_3 - D_2)}.$$

ESTIMATION OF THE NUMBER OF BLOOD CELLS.

The Hematocytometer. The instrument now in use for counting blood cells is that of Thoma, who combined and improved several features of instruments previously devised by Hayem, Gowers, and Malassez. This apparatus consists of a mixing pipette and a counting-chamber.

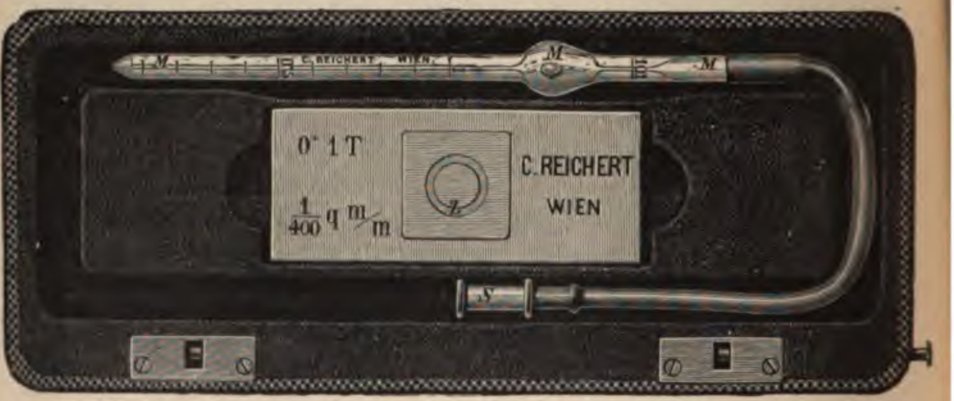
(*a*) **The pipette** is a capillary tube graduated in ten equal divisions, surmounted by a bulb of exactly 100 times the capacity of the tube, and to which is attached a rubber tube and mouth-piece (Fig. 7). When the tube is filled with blood up to the mark 1, and this is mixed with a diluting fluid sucked up to the mark 101, a specimen of blood is obtained in the dilution of 1 : 100. By filling only one-half the tube with blood, up to the mark 0.5, the subsequent dilution is in the proportion of 1 : 200. The bulb contains a glass ball to facilitate the mixing of the blood.

(*b*) **The counting-chamber** is constructed so as to secure a layer of diluted blood $\frac{1}{10}$ millimetre in depth over a certain square area.

On a thick glass slide is cemented a thinner glass plate, the central portion of which is cut out. In this central area is cemented a circular glass shelf the surface of which is exactly $\frac{1}{10}$ millimetre lower than the surface of the glass plate. When a drop of diluted blood is placed on the shelf and covered with a cover-glass, a layer of fluid is secured, which is exactly $\frac{1}{10}$ millimetre deep. Between the edge of the shelf and the surrounding plate is a moat into which the blood may run, but if the fluid should run over the moat and beneath the cover-glass, the latter will be elevated and the resulting layer of fluid will be more than $\frac{1}{10}$ millimetre deep. The shelf is accurately ruled, as shown in Figs. 8 to 11.

The entire ruled area is nine square millimetres, but only the central square millimetre is used in counting red cells, the others being required in counting leucocytes. It will be seen that this central square millimetre is subdivided into 400 small squares (16 blocks of 25 each), so that each small square is $\frac{1}{400}$ sq. mm. Beginning at the lower left-hand corner of this area, it will be seen that every fifth square, above and to the right, is subdivided by an extra line, which is added merely to assist in counting the squares. The outlying square millimetres are variously ruled.

FIG. 7.



The Thoma hematocytometer.

The above description applies only to the so-called "Zappert" chamber which should always be secured, preferably of Zeiss' manufacture. The older chambers cannot well be used for counting leucocytes.

Diluting Fluids. Of the various diluting fluids, TOISSON'S MIXTURE is to be recommended:

Sodium sulphate	8.0 grm.
Sodium chloride	1.0 "
Glycerin pur.	30.0 "
Aq. dest.	160.0 "
Methyl violet	0.25 "

This fluid keeps well, stains the leucocytes, and is of high specific gravity so that the red cells settle from it slowly.

When counting leucocytes only, one may use with advantage a 0.6 per cent. solution of sodium chloride tinged with gentian violet (about 1 drop of saturated alcoholic solution gentian violet to 50 c.c. of salt solution). This fluid, while readily prepared, does not keep well, and the red corpuscles settle from it so rapidly that it ought not to be used in counting these cells. It permits, however, of the identification of eosinophile cells and of certain degenerative changes in leucocytes.

A reliable fluid for diluting and permanently preserving blood is found in HAYEM'S MIXTURE:

Hydrarg. bichlor.	0.5 grm.
Sod. sulphat.	5.0 "
Sod. chlor.	1.0 "
Aq. dest.	200.0 "

Directions for Using the Hematocytometer. (*a*) FILLING THE PIPETTE. The finger-tip of the patient is cleansed with soap and water, dried with alcohol, and freely punctured with a needle or a specially prepared acne-lancet. Using very gentle pressure only, a compact drop of blood is then expressed and the capillary tube is filled to the mark 1 or 0.5. In doing this the pipette must be held between the thumb and forefinger and the hand steadied against the hand of the patient. In well-constructed pipettes the column of blood is easily controlled, and after filling the end of the tube may be cleansed of adherent blood. The diluting fluid is then sucked up to the mark 101, taking care that no blood runs out of the tube when it is immersed in the fluid. The specimen is then thoroughly mixed by shaking.

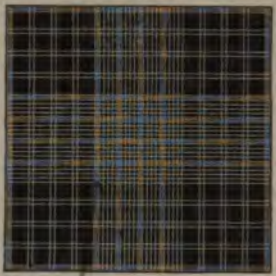
(*b*) FILLING THE COUNTING-CHAMBER. The counting-chamber and cover-glass are thoroughly dried and freed from particles of dust. One or two drops of diluted blood are first forced from the pipette, and a third drop, the size of which can be learned only by experience, is deposited on the central shelf. The cover-glass is then immediately adjusted, slipping one corner under the forefinger of the left hand and controlling the opposite corner with the second finger of the right hand, and lowering the glass slowly so as not to include air bubbles. Without raising the fingers, now quickly cover the other corners with the forefinger of the right and second finger of the left hand, and press the cover-glass firmly into position. If the application is successful and no dust particles have intervened, *Newton's color rings* will appear beneath the cover-glass. The formation and permanency of these rings may be facilitated by breathing *very gently* on the specimen before applying the cover-glass.

The specimen should now be held up to the light and examined closely to see that the red cells are evenly distributed. An uneven distribution is readily detected by the naked eye. After settling a few moments the specimen is ready for counting.

The rapid and successful adjustment of the cover-glass is the most important detail in the process of counting blood cells. The cover-glass must be *rapidly* adjusted, because from the moment the drop is placed upon the shelf there is a rain of cells upon the ruled area out of a layer of fluid which is more than $\frac{1}{10}$ mm. deep.

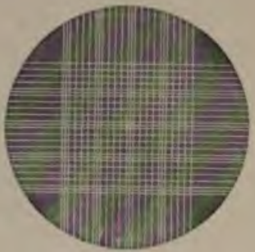
The specimen must be discarded:
If Newton's rings do not appear.
If any air bubbles are inclosed.
If the fluid runs underneath the cover-glass.
If the shelf is not well covered by fluid.
If, on inspection, the cells are found unequally distributed.

FIG. 8.



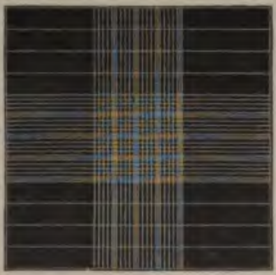
Türck.

FIG. 9.



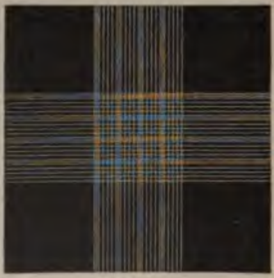
Thoma. Centre part.

FIG. 10.



Zappert-Ewing.

FIG. 11.



Thoma.

Blood-counting chambers.

(*c*) COUNTING THE RED CELLS. The specimen proving satisfactory, the count may begin as soon as the cells have settled. Zeiss, D., Leitz, No. 7, Reichert's or Bausch and Lomb, $\frac{1}{6}$, are the lenses best suited for this purpose, and a good mechanical stage is necessary for accurate work. Locate in the field the lower left-hand block of 25 small squares, begin at the lower and left square and passing to the right count all the cells lying in the first five squares. The fifth square will be found subdivided. In each square count all the cells lying on the *lower* and *left* side lines, leaving to be counted with the adjacent squares all the cells lying on the lines *above* and to the *right*. Proceed in this way until at least four blocks of 25 small squares and at least 1000 cells are enumerated. The more squares counted over the greater the accuracy, and when slight variations are to be demonstrated the entire square millimetre must be covered. If the cells now appear to be unevenly distributed the specimen should be discarded and another prepared after thoroughly shaking the pipette.

(*d*) COMPUTATION. Suppose that 1280 cells are enumerated in 100 small squares, *i. e.*, in $\frac{1}{4}$ of the square millimetre. This number multiplied by 4 gives the number lying over one square millimetre. But the depth of the fluid is only $\frac{1}{10}$ mm., so that we multiply again by 10 to get the number of cells in one *cubic* millimetre of fluid. Finally we must multiply by 100 because the blood is *diluted* in the proportion of 1 : 100.

In short, after counting over 100 small squares the result is multiplied by *4000* to give the number of cells per cubic millimetre ($4 \times 10 \times 100 = 4000$).

If the capillary tube was originally filled to the mark 0.5, the dilution is 1 : 200, and the multiplier *8000*. If 400 squares are counted over, the multiplier is *1000*.

(*e*) SOURCES OF ERROR IN THE USE OF THE HEMATOCYTOMETER. 1. *In Securing the Drop of Blood.* When much pressure is employed in expressing the drop of blood, tissue fluids are squeezed out with the blood and the number of red cells is reduced. Reinert found a reduction of 722,000 from this cause, which is especially potent in cases of dropsy and of severe anemia.

When the finger is cold, the circulation poor, or local stasis is produced, as by a ligature, the red cells are increased in number. To avoid errors of this class, the circulation in the hand should be as active as possible, the finger warm, and the puncture liberal enough to permit the flow of blood with little pressure applied at some distance from the puncture. Unless these conditions can be secured it is hardly worth while to count the blood cells.

2. *In diluting the blood and in transferring it to the counting-chamber* there are numerous plainly evident sources of error, such as the inaccurate filling of the capillary tube, the entrance of air with the blood column, the failure to cleanse the tip of adherent blood, the escape of blood into the diluting fluid, the overfilling of the bulb with diluting fluid, the inadequate mixture of the blood, the failure to discharge one or two drops before applying one to the shelf, the use of thin cover-glasses, and, above all, delay and inaccuracy in adjusting the cover-glass. A little experience and constant care serve to eliminate all these difficulties.

3. *In the Construction and Condition of the Apparatus.* The tendency to favor the Zeiss instruments is still probably well founded, although Leitz and Reichert are now making very excellent pipettes after Grawitz's model. Aside from inaccuracies in the graduation of the pipette and construction and ruling of the counting-chamber, which are now reduced to a minimum, some pipettes are still on sale which are too short, their calibre is too large, and is narrowed at the point so that the tubes cannot be cleaned, they require too much blood, and the short arm is so small that the mark 101 comes too close to the bulb. Accurate work cannot be performed with such instruments. The worker is at present advised to insist on having Grawitz's pipette made by Zeiss, Leitz, or Reichert.

Many close observers find that their pipettes vary with the temperature. While accurate information on this point is not at hand it is just as well to avoid extremes of temperature in making the tests and in cleaning the instrument. It has been suggested that the polycythemia of high altitudes is partly referable to variations in the hematocytometer due to changes in atmospheric pressure, but this suspicion has not been confirmed.

The condition of the pipette is of prime importance. Absolute dryness of the tube and bulb is essential. The collection of minute water drops in the tube and bulb is responsible for many of the shadow corpuscles sometimes seen in the counting-chamber.

Every few weeks a pipette should be cleaned out with concentrated nitric acid.

(*f*) CLEANING THE APPARATUS. After using the pipette the rubber tube may be transferred to the long arm and the remaining fluid expelled. The tube should then be cleaned thoroughly with water, then with alcohol and ether, or, better, with pure ether. It must be thoroughly dried before using again. The counting-chamber must be cleaned with *water only*, as *alcohol and ether dissolve the cement under the shelf and plate.*

(*g*) THE LIMIT OF ERROR WITH THE HEMATOCYTOMETER. Lyon, Thoma (and Reinert), counting an average of 1141 cells in 100 squares with a dilution of 1 : 200, found an average error of 1.82 per cent. in 24 preparations of the same specimen, and in another case, counting an average of 934 cells in 100 squares, 1 : 200 dilution, an average variation of 2.71 per cent., in 12 preparations of the same specimen (Limbeck). These results in the hands of experts using special care indicate that a variation of 150,000 cells (3 per cent.) cannot be accepted as of any significance. More accurate data are, however, seldom required by the clinician.

Oliver's Hematocytometer. **The Principle.** When a candle flame is viewed through a flat glass tube containing water a transverse line of bright light is seen which results from the blending of numerous images of the flame. The images are produced by the minute longitudinal corrugations in the glass which reflect the light in various directions. When diluted blood is placed in the tube the fluid is sufficiently opaque to shut out the images until a certain dilution is reached, when a bright streak of light becomes rather suddenly visible.

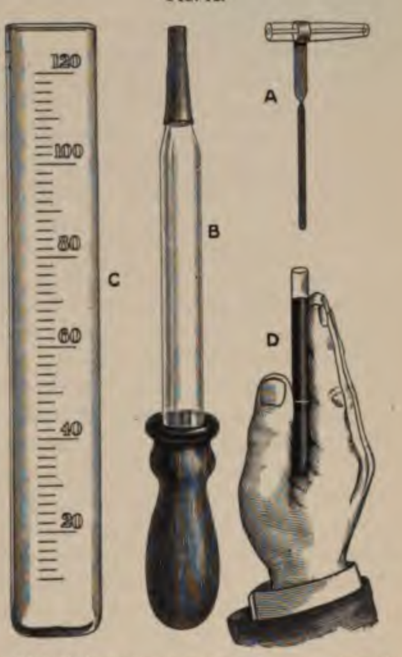
Oliver believes that the appearance of this bright horizontal line is a very sensitive indicator of the proportion of red cells in the fluid, and by a long series of observations has devised an instrument for determining the number of red cells in blood according to this principle.

The apparatus consists of a measuring pipette (A); a dropper for Hayem's fluid (B); a flat glass tube graduated in 120 degrees (C).

The Procedure. The capillary pipette is carefully filled with blood and washed into the tube by means of Hayem's fluid. A proper amount of fluid is then added to the diluted blood, and the two are mixed by inverting the tube closed by the thumb, care being taken not to remove any diluted blood with the thumb. The test should be made in a dark room, the light being furnished by a Christmas candle placed about ten feet from the operator. When the blood is insufficiently diluted the image of the candle is invisible when looking through the tube held horizontally (Fig. 12, D), but at a certain dilution the images begin to appear, and at the proper dilution a rather compact transverse line of light becomes visible. The bottom of the meniscus is then read off on the graduated scale. Each degree of the scale represents 100,000 red cells, the mark 100 corresponding to 5,000,000 cells, 80 to 4,000,000, 60 to 3,000,000, etc.

There are both theoretical and practical objections to the use of Oliver's instrument. Theoretically, the method falls in the undesirable class of indirect methods about which there are always a large series of unknown disturbing factors which can only be eliminated by prolonged experience.

FIG. 12.



Oliver's hematocytometer. A, measuring pipette. B, dropper. C, mixing tube graduated in percentages. D, mode of observation.

Baumgarten finds that in addition to the variation in size of the corpuscles an important source of error is the coagulation of the serum by the Hayem's solution. He found variations of 20 to 30 per cent. from the results obtained by Thoma's instrument, and concludes that the method is of no value in clinical work. Scannell's report, however, is more favorable.

Practically the difficulty of determining the exact dilution from the appearance of a compact line of light is very great. The method cannot be recommended until it has received much wider application than it has yet enjoyed.

The Estimation of Leucocytes.

The leucocytes may be counted by a method which requires a special mixing pipette, yielding a dilution of blood in the proportion of 1 : 10, and a diluting fluid (3 per cent. acetic acid, tinged with gentian violet) which dissolves the red cells, leaving only the stained leucocytes to be counted. The same chamber is used as for counting red cells, and the same procedure is followed. All the leucocytes in one square millimetre having been counted, the result is multiplied by 100, giving the number of leucocytes per cubic millimetre.

The disadvantages early recognized in this method are the expense and inconvenience of an extra pipette, and a second diluting fluid, the time required in preparing a second specimen, the larger quantity of blood required, the difficulty sometimes encountered in distinguishing leucocytes from the detritus of red cells, and the impossibility of separating and evenly distributing the cohesive leucocytes.

This method has gradually been replaced to a large extent by the practice of counting leucocytes in the same specimen prepared for counting the red cells. In 1892 the writer found that he secured more uniform results with the latter method, and has since found no inducement to return to the former.

The Counting of Leucocytes in the Same Preparation with the Red. This method requires the ZAPPERT CHAMBER, which was originally devised by Elsholz for the estimation of eosinophile cells in fresh blood. Varous modifications of the ruling in this chamber have been employed, one of which, made by Leitz, at the writer's suggestion, is represented in Fig. 10.

With this chamber, using a Leitz lens No. 7, it is possible to count over nine square millimetres, which gives almost as many leucocytes as are counted in the other method. When the leucocytes are normal or reduced in number, it is necessary to count all there are in the available 9 sq. mm., and if the number is very low it is advisable to prepare a second specimen in the chamber and count the white cells in 18 sq. mm. When the leucocytes are increased, 9 sq. mm., or in cases of leukemia, 6 sq. mm., will yield a number large enough to insure an accurate result.

In order to make the leucocytes visible the Toisson's fluid or other solution should contain enough methyl violet to stain these cells distinctly. With a little practice the eye very readily picks out the bluish highly refractive leucocytes.

What has been said regarding the condition of the local circulation, and the effects of pressure in expressing the blood, is to be specially emphasized when estimating the number of leucocytes in a specimen of blood.

Computation. Divide the number of leucocytes counted by the number of square millimetres traversed in the count and multiply by 1000. The result is the number of leucocytes per cubic millimetre of blood.

If the original dilution is 1 : 200, which ought not to be employed except in cases of leukemia, the multiplier is 2000. Thus if 54 leucocytes are counted in 9 sq. mm. (dilution 1 : 100) the number per cubic mm. is 6000 (54 ÷ 9 × 1000).

The Enumeration of Eosinophile Leucocytes. (*a*) **In the Same Preparation with the Red Cells.** When the blood is diluted, 1 : 100, with 0.6 per cent. salt solution tinged with gentian violet, the leucocytes retain their natural size and shape and eosinophile cells can be readily identified by their large, greenish, refractive granules. In cases of myelogenous leukemia this method is satisfactory, but when the eosins are present in their usual numbers (1 to 5 per cent.) one must count a larger number than can be found by this method. The usual expedient is to estimate their percentage from a dried specimen of blood, and then to calculate their number from the total number of all leucocytes counted by other methods. Thus, if the count shows 12,000 leucocytes per cubic mm. and the dried blood slide shows 2 per cent. of eosins their number will be 240 per cubic millimetre. This method is sufficiently accurate for clinical purposes.

(*b*) **By Means of Thoma's Special Pipette for the Enumeration of Leucocytes.** Klein, Mueller and Reider, and Elsholz, have employed methods for the accurate estimate of eosinophile cells adapted to finer clinical work and to experimental research. They use the large pipette of Thoma which gives a dilution of 1 : 10. The capillary tube is filled with blood to the mark 1, and the bulb is half filled with the following solution : watery eosin (2 per cent.), 7 c.c. ; glycerin, 45 c.c. ; aq. dest., 55 c.c. After shaking three to four minutes the bulb is filled to the mark 11 with the following staining fluid : aq. dest., 15 c.c. ; gentian violet, conc. aq. sol., 5 drops ; alcohol, 1 drop.

In specimens thus prepared both neutrophile and eosinophile leucocytes are readily distinguished, the eosinophile cells being particularly brilliant. The red cells are dissolved and the leucocytes concentrated so that a sufficient number of eosinophile cells may be counted.

Zappert's extensive studies of eosinophile leucocytes were conducted with specimens diluted in the large pipette of Thoma, by the following solution : 1 per cent. osmic acid sol., 5 c.c., to which are added 5 drops of a filtered mixture—aq. dest., 10 c.c. ; glycerin, 10 c.c. ; 1 per cent. watery eosin, 5 c.c.

THE ESTIMATION OF HEMOGLOBIN.

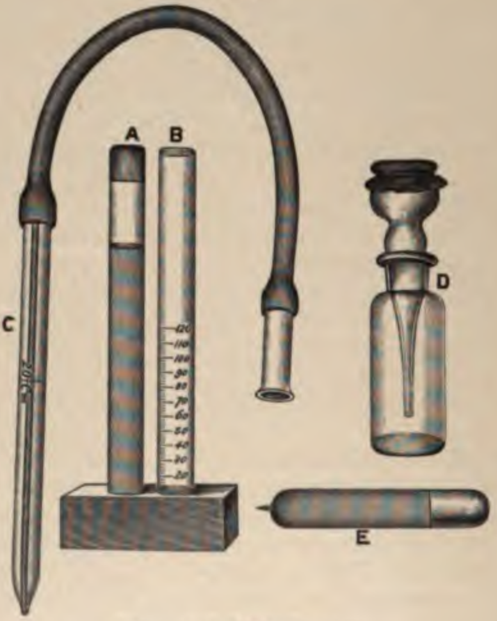
1. **Gowers' Hemoglobinometer.** This instrument has always been largely employed on account of its cheapness and simplicity, and the ease and rapidity with which its results are obtained. Except with low percentages of Hb, it is tolerably accurate, but much less reliable than Fleischl's instrument. With low percentages of Hb it is well to use a double quantity of blood, halving the result.

Aside from any inaccuracy in the construction of the apparatus, errors arise chiefly from the difficulty of adding exactly the proper quantity of water to the blood, and the imperfect comparison of red colors in daylight. The apparatus consists of two glass tubes (A, B) of exactly equal calibre, one of which is partly filled with gelatin colored by picrocarmine so as to represent the color of a 1 per cent. solution of normal blood. The second tube carries a graduated scale from 10 to 120 and serves to hold the diluted blood. The capillary pipette C measures 20 c.mm., the quantity of blood to be used.

In making the test the pipette is filled to the mark 20 c.mm. with blood obtained under the usual precautions. The specimen is quickly discharged into the tube B, in which a few drops of distilled water have previously been placed. The pipette must then be washed once or twice into the tube, the distilled water removing all traces of blood adherent to the inside of the capillary tube. Distilled water is now added drop by drop, until the solution of blood, carefully shaken and mixed, exactly matches the carmine gelatin. The percentage of Hb is indicated by the height of the solution on the scale, reading from the middle of the meniscus. The colors match in daylight, and the eye may be assisted by holding the tube in front of white paper.

Haldane's Modification. The picrocarmine solution of Gowers' instrument is not stable and has been replaced by Haldane with a 1 per cent. solution of CO-Hb, which he has found to remain unchanged for months, even when exposed to heat and sunlight. The specimen of blood is drawn as usual and partly diluted. The air in the tube is then expelled by a current of illuminating gas introduced from a gas-burner by a rubber tube, and CO-Hb formed by inverting the tube, closed by the thumb, several times. The dilution and reading are then completed. Haldane claims very accurate results from this method. The apparatus with standard solution of CO-Hb is supplied by the makers of Gowers' hemoglobinometer.

FIG. 13.



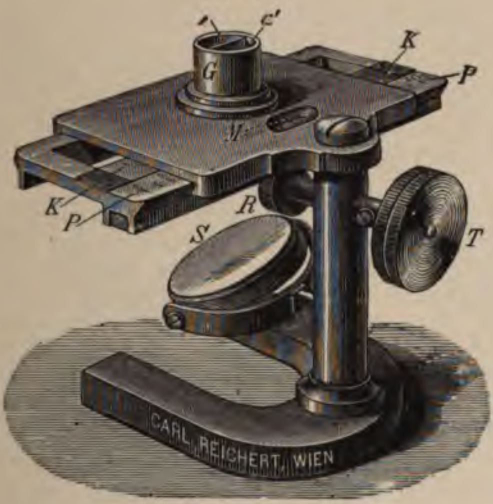
Gowers' hemoglobinometer.

2. **Tallquist's Method of Estimation of Hemoglobin.** Tallquist has recently described a rough method of estimating hemoglobin by comparison of a piece of filter paper stained by the blood with a carefully prepared lithographic plate representing the colors of ten solutions of Hb, ranging from 10 to 100 per cent. A small piece of white filter paper of standard quality, such as is sold with the scale, is touched to a rather large drop of blood, which is allowed to diffuse

slowly through the paper, so as to give as even a stain as possible. Immediately after the stain has lost its lustre and before drying or changing color by exposure the percentage of Hb is determined by matching with one of the colored areas in the scale. It is unnecessary to mention the serious sources of error in this method, which hardly estimates but rather assists one to judge the percentage of Hb.

3. **Fleischl's Hemoglobinometer.** (*a*) **Apparatus.** This apparatus consists of a metal stand with plate, and plaster mirror (*S*) which casts diffused light through a circular opening in the plate. Beneath the plate, by means of a rack and wheel (*T*), slides a colored glass wedge fixed in a graduated frame (*P*). The glass wedge and graduated scale are arranged so as to indicate the percentage of Hb corresponding to the different portions of the wedge. In the circular opening of the plate fits a cylindrical metallic cell (*G*), with glass bottom and metal partition, one compartment of which lies directly over the glass wedge. The other compartment (*a*) being filled with diluted blood, one is able to make a close comparison of the color of the dissolved blood with that of the glass wedge. The blood is measured by an automatic capillary pipette, while a slowly running dropper is provided with which to add distilled water. On the handle of each pipette is stamped a number, indicating the cubic content of the tube. On the stand of each instrument is also a number showing the capacity of the tubes with which it can be used.

FIG. 14.



Fleischl's hemoglobinometer.

(*b*) **Procedure.** One should first see that the automatic pipette is in working order, by blowing it out several times with water until it fills instantly and completely, after which it must be thoroughly dried. A drop of blood having been expressed under very strict precautions against pressure, one end of the pipette is lightly touched to the drop which instantly fills the tube. There should be neither negative nor positive meniscus to the column of blood, but the tube should be level full at either end. It should not be immersed in the blood drop, otherwise blood will adhere to the sides which cannot safely be removed. The tube of blood is immediately transferred to one compartment of the cell which has been half filled with distilled water, and the blood is thoroughly dissolved by moving the tube rapidly from side to side. On withdrawing the tube it should be washed into the cell with a few drops of distilled water. With the handle of the pipette the blood which collects in the corners of the chamber is thoroughly mixed. Both chambers of the cell may now be filled level full with water. The thick round cover-glass should then be adjusted, avoiding the inclosure of air. Serious error may here result by forcing dissolved blood over into the water compartment or over the side of the cell. If the reading is made promptly the cover-glass need not be used, but after an exposure of ten to fifteen minutes the oxidizing action of the air may darken the blood and increase the reading 10 to 15 per cent.

The reading must be done in a dark room by means of candlelight or gaslight. The colors do not match in daylight. The best results are obtained by placing the candle about eighteen inches from the stand, and by looking through an improvised paper tube which exactly fits the cell. *With low percentages of Hb a very dim light is essential.* When the cell is in place, and the light adjusted, the wedge is moved with quick rather than gradual turns until the color of the glass exactly matches that of the dissolved blood, when the percentage of Hb may be read on the scale. Several expedients are employed to assist the eye in the comparison of colors. It is well to relieve one eye with the other so as not to exhaust the color sense. The strength of the light may be varied by altering its distance, but the faintest distinct light is usually found to be the best. Two or three readings should always be made and the average taken. As the graduation of the instrument is more accurate and the matching of colors more exact in the middle of the scale, it is advisable to use a double quantity of blood when dealing with low percentages of Hb.

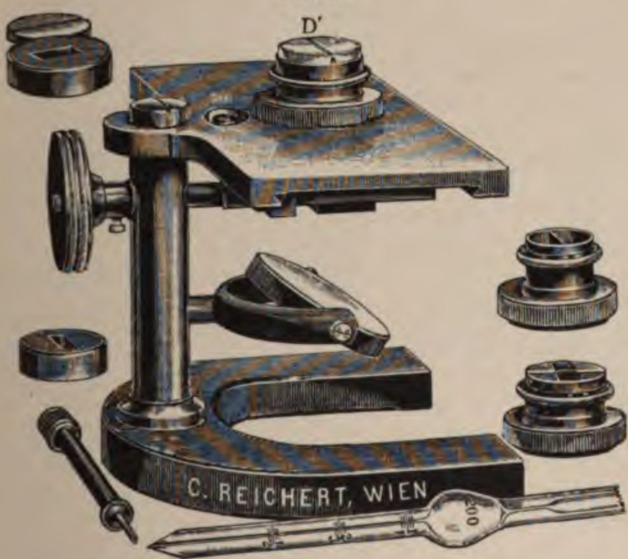
(*c*) **Limitations of Fleischl's Instrument.** With considerable experience and constant care Fleischl's instrument yields results which are sufficiently accurate for most clinical purposes. One cannot attach any significance, however, to a variation of 5 per cent., within which figure the ordinary error ought to be limited.

All the causes of error mentioned as affecting the number of red cells may also disturb the percentage of Hb in the blood drop. In the procedure the principal difficulties relate to the even filling of the tube, the thorough cleaning of the tube, the overflow of blood solution into the adjoining chamber, the proper adjustment and regulation of the light, and the accurate matching of colors.

The chief sources of error in this method probably lie in the construction of the instrument. It is a common experience to find that different instruments give different results with the same specimen of blood, which Limbeck satisfied himself were referable principally to differences in the glass wedges.

The writer tested his own blood with sixteen different instruments, two of which were old (1892), the others of more recent importation (1897). With the two old instruments the Hb registered 87 per cent. and 90 per cent.; with the newer instruments it varied between 97 per cent. and 105 per cent., except with one which gave 85 per cent. with different cells and different capillary tubes. The colored wedge with this instrument was broader and darker than usual, and when it was replaced by another wedge the instrument registered 100 per cent. with the same specimen. The error attributable to variations in different tubes and cells the writer finds is seldom greater than 5 per cent. among the newer instruments. Many old instruments read 10 to 15 per cent. lower than normal.

FIG. 15.



Miescher's hemoglobinometer.

The possible combination of these various defects in construction renders it desirable that one should test every new instrument for himself, using the blood of five or six healthy subjects. The newer instruments will not be found to require much correction.

Miescher's Modification of Fleischl's Hemoglobinometer. Miescher's improvements have removed some important defects in Fleischl's instrument, and as it now stands the improved hemoglobinometer yields results that leave little to be desired in point of accuracy.

With this apparatus the blood is diluted by means of a graduated pipette very similar to that of Thoma, but yielding dilutions of 1 : 200, 1 : 300, and 1 : 400, according as the tube is filled with blood to the mark $\frac{1}{1}$, $\frac{2}{3}$, or $\frac{1}{2}$.

Two cells are provided, one with a depth of 15 mm., the other with a depth of 12 mm., the percentage of Hb being obtained with the deeper cell, and the other being used as a control specimen and giving only $\frac{4}{5}$ ($\frac{12}{15}$) of the actual percentage of Hb.

These cells have a *projecting partition* dividing the compartments, along which a grooved cover-glass (D) may be slid, without fear of mixing the blood and water. If an excess of the blood solution overflows the side of the cell during the adjustment of the cover, no harm results, as the blood is already properly diluted in the pipette, and the depth in the chamber will always be 15 mm. (or 12). These changes add very much to the facility and precision of the method.

Finally, the cells are covered with diaphragms transmitting a ray of light which includes only three degrees on the scale, thus giving practically a single color of the wedge for comparison with the blood.

In the procedure the blood is diluted in the pipette as with the hematocytometer, the diluting fluid being distilled water or a filtered 1 per cent. solution of sodium carbonate. After shaking and clearing the tube of diluent, one chamber in each cell is filled with diluted blood, the opposite chamber with distilled water, and cover-glasses and diaphragms are adjusted. Using a small candle and shielding the eyes from light, the readings with the two cells are carefully taken. The reading with the small cell should be $\frac{4}{5}$ that with the larger. If there is any variation one reading may be used to correct the other. For example, suppose the readings to be:

For the larger chamber (15 mm.)	64
For the smaller chamber (12 mm.)	50

If the first reading were absolutely correct the second reading should have been 51.2, since $64 \times \frac{4}{5} = 51.2$. Or, assuming the second to be correct, the first should have been 62.5, since $50 \times \frac{5}{4} = 62.5$. The mean of 64 and 62.5, *i. e.*, 63.25, should be taken as the true value.

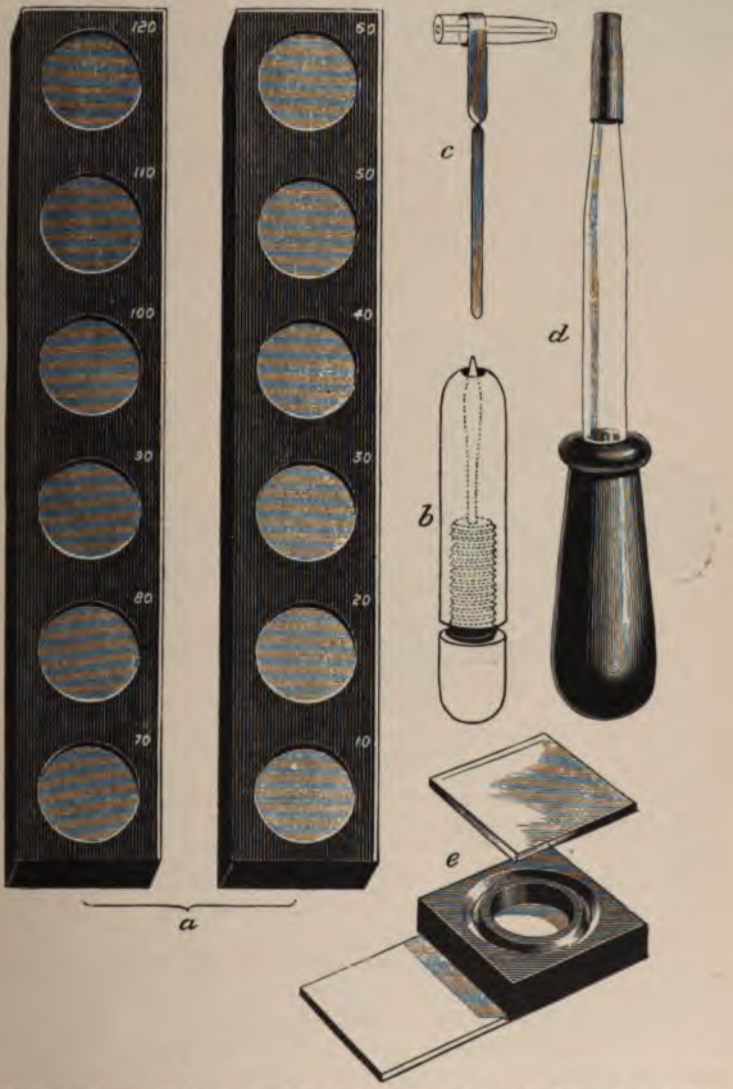
If the original dilution was 1 : 200, the percentage of Hb is 63.25, the corrected result with the larger cell, but if the dilution has been 1 : 300, this result must be multiplied by 1½, or if 1 : 400, by 2.

The technical difficulties of this method are so slight and the results so accurate that the instrument may be recommended over any other yet devised for this purpose.

4. **Oliver's Hemoglobinometer.** This instrument is constructed on the excellent principle of the tintometer, which is extensively used in various arts and industries.

It consists of a series of six red glass disks (*a*) mounted upon white plaster mirrors in convenient frames. These disks represent the colors of twelve solutions of blood containing twelve different proportions of Hb. Two sets of disks are made, one for reading in daylight, the other for candlelight. The latter give more accurate results and should be chosen. The intermediate percentages of Hb are secured by means of "riders" of colored glass to be placed over the disks, and which represent respectively 2½ and 5 per cent. of Hb in the upper half of the scale, but twice that amount in the lower half. The disks are graded according to the specific dilution-curve of Hb.

FIG. 16.

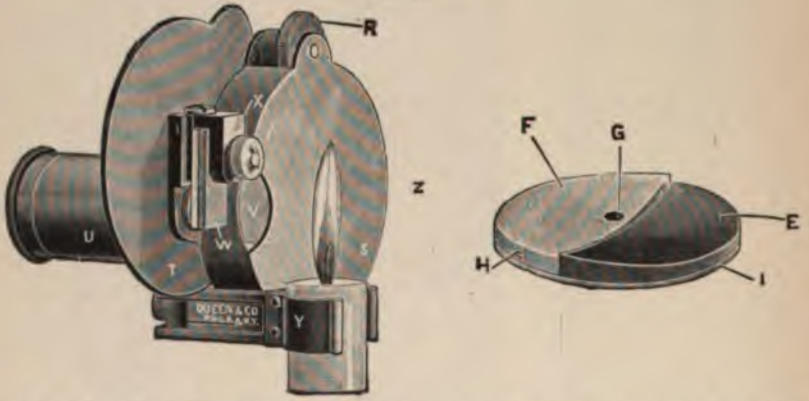


Oliver's hemoglobinometer. *a*, set of standard colored disks. *b*, lancet. *c*, capillary pipette. *d*, dropper. *e*, mixing-chamber.

The blood is measured in an automatic pipette, *c*, and diluted in a cell, *e*, provided with a white plaster, glass-covered bottom, and blue glass cover, which facilitates the reading. The blood is washed from the tube by means of a pipette, *d*, with rubber nozzle. The tube may be cleaned by means of a thread and needle.

Procedure. The capillary tube is filled by touching it to the blood drop, and the blood is washed into the cell by attaching the rubber nozzle of the pipette, filled with distilled water, taking care not to aspirate any blood into the pipette. The handle of the tube is then used to mix the blood, and water is carefully added until the cell is level full. The cover is then applied and should inclose a minute air bubble, showing that the chamber has not been overfilled. The comparison of colors should be made by candlelight, the candle being placed at a convenient distance from the disks, and the eye shielded by means of a hood or paper tube. Oliver uses a special camera with green-glass eye-piece. If the color of the specimen matches any one of the disks the reading is completed. If it does not, the disks may be varied by using the "riders," adding a clear glass disk to the specimen to compensate for the thickness of the "rider."

FIG. 17.



Dare's hemoglobinometer (× ½). Parts in position. R, milled wheel rotating colored disk and scale. S. case inclosing disk. T, shield which receives the camera tube U V, visible portion of disk backed by white glass. W, white glass back of capillary chamber. Y, candle holder. Contents of case: E, colored disk. F, clear glass disk graduated along edge, H. I, white glass back. G, opening for pivot.

Oliver's instrument presents the advantage of a comparison with a single color instead of with a scale of 10 per cent. +, as in Fleischl's. This difficulty is slight, however, and is overcome in Miescher's modification of Fleischl's instrument. The color of Fleischl's glass wedge varies according to the specific dilution-curve of the colored glass, which differs from the specific dilution-curve of Hb. How serious an error may arise with Fleischl's instrument from this cause has not been clearly shown. It is probably not great, as it is not apparent in the use of Miescher's modification in which different parts of the scale are employed. Yet the principle of Oliver's instrument is, in this respect, a distinct improvement.

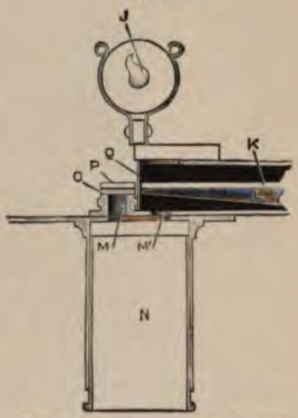
The technical difficulties are considerably less with Miescher's hemoglobinometer.

5. **Dare's Hemoglobinometer.** This instrument consists of the following parts: 1. A capillary blood chamber composed of two rectangular pieces of glass which when clamped together leave a thin space at one end into which a layer of blood flows by capillary attraction. One of the plates is of porcelain glass, which diffuses the light.

This chamber, when filled and adjusted, lies over one of two apertures which meet the eye glancing through the camera tube of the instrument.

2. The color standard is a semicircular glass plate stained with Cassius' golden purple. The plate varies in thickness, giving colors which have been accurately adjusted to match various percentages of Hb. This plate is inclosed in a rubber case in which it rotates by means of a milled screw, carrying with it another glass plate with scale adjusted from 10° to 120°, which may be read on the left of the rubber case.

FIG. 18.



Dare's hemoglobinometer. Horizontal section. Capillary chamber and clamp. J, candle. K, white glass back. L, colored disk. M, opening through which blood is viewed. M', opening in colored disk. N, camera tube. O P, clear and white glass plates of chamber between which lies the layer of blood. Q, metallic septum.

3. A detachable camera tube of black rubber screws into a movable shield attached to the case, and when the parts are in position this tube furnishes a dark chamber through which the operator compares the color of the blood with that of the standard scale, each of which is exposed by small circular openings in the shield. The light is provided by a candle fixed to the case. The wick should be trimmed low.

Method of Use. One edge of the capillary chamber, firmly clamped, is touched to a rather large drop of blood sufficient to fill it rapidly and completely, after which the chamber is slipped into the holder, white glass outward.

Rotating the shield and camera tube outward and pointing the instrument toward a dark background, one finds the color scale and blood film clearly outlined in the camera tube, and by rotating the milled wheel the color scale is brought to match the blood. The instrument should be held steady, so that the flame does not flicker, and the reading should be completed promptly before the blood coagulates or evaporates.

Dare's instrument is very cleverly devised and constructed. It requires the use of pure blood, offers ideal conditions for the comparison of colors, and demands little space in transportation, little time in application, and little pains in cleansing, while in accuracy of results it stands next, but inferior, in the author's experience, to the Miescher-Fleischl apparatus.

ESTIMATION OF THE IRON OF THE BLOOD.

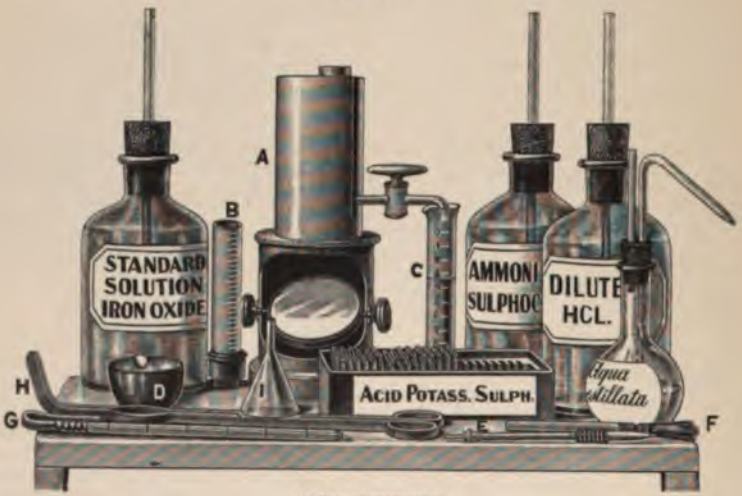
Jolles' Ferrometer. Jolles has devised a method and designed apparatus for the estimation of the iron of the blood, which is well adapted to clinical purposes.

The apparatus is manufactured by Reichert, and full directions in German accompany each set.

The procedure is as follows:

By means of a pipette 0.05 c.c. of blood is transferred to a platinum crucible, and the adherent blood washed out with a few drops of water. The blood is then evaporated and incinerated over a Bunsen flame. The ash is melted with 0.1 gr. water-free potassium bisulphate, until white fumes of sulphuric anhydride cease to rise from the dish.

FIG. 19.



Jolles' ferrometer.

Papers containing the requisite amount of potassium bisulphate accompany the instrument. After cooling, the ash is washed into cylinder C (Fig. 20) with about 5 c.c. of hot distilled water, which is added until the whole quantity is 10 c.c.

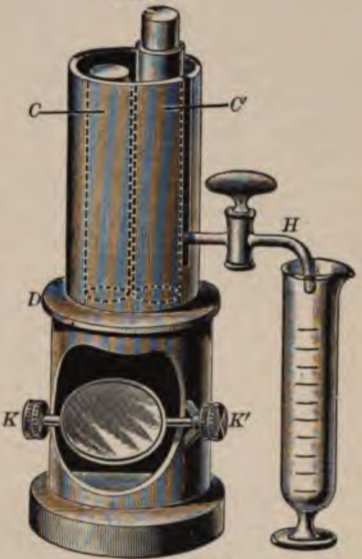
In cylinder C' 1 c.c. of the standard solution of iron (0.00005 gr. iron oxide with potassium sulphate) is measured by a pipette and distilled water added to the mark 10 c.c. Both cylinders are now placed in the stand (Fig. 20), and when at even temperature 1 c.c. of dilute HCl (33 per cent.) is added to each.

To cylinder C' should then be added 4 c.c. of the solution of ammonium sulphocyanide, and to cylinder C about 3.5 c.c., and both are shaken after covering the ends with glass plates.

Cylinder C, containing the blood, is now filled with sulphocyanide until it presents a positive meniscus, when it is permanently covered and sealed by its glass plate. It may then be placed in the colorimeter. In cylinder C' the aluminum float is then adjusted, free from air bubbles, and it is also placed in position in the colorimeter.

The further procedure consists in bringing the color of C' to match that in C, when viewed from above in the colorimeter. For this purpose the fluid in C' is allowed to run out drop by drop through a stopcock until the two colors are exactly alike. The comparison should be made in daylight. When the colors exactly match the height of the necessary column of fluid in cylinder C' may be read off and the percentage weight of iron determined by reference to a table accompanying the instrument. The Hb may then be found according to v. Jaksch's formula,

FIG. 20.



Jolles' ferrometer.

$$\text{Hb} = \frac{100 \times m}{0.42}$$

in which m = the percentage weight of metallic iron.

In order to secure accurate results with the ferrometer it is necessary to observe the same care that is required in all quantitative chemical analyses, especially to avoid the loss of fluids by sputtering from the hot crucible, the use of wet and unclean instruments, and the inclosure of air bubbles, etc. Under most conditions the Hb may be accurately determined by means of the above formula, but from the considerations mentioned under "The Occurrence of Iron in the Blood," it will be seen that there may be considerable variations between the iron and the Hb-content. The ferrometer, therefore, usually gives a higher proportion of Hb than does Fleischl's instrument.

THE HISTOLOGICAL EXAMINATION OF BLOOD.

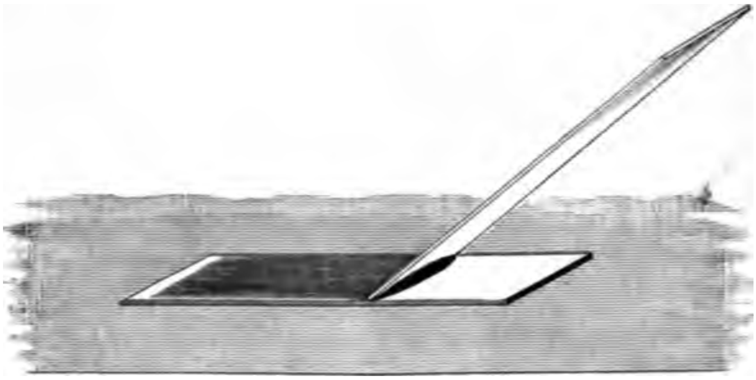
The greater part of the examination of blood is conducted with dry stained specimens. To prepare such specimens for staining one requires only polished glass slides and a Bunsen gas-burner. The glass slides must be thoroughly cleaned with soap and water, dried, and kept free from dust. Passing them a few times through a flame facilitates the even spreading of the cells.

A rather small compact drop of blood expressed from the finger tip under the usual precautions is lightly scraped off with the polished edge of one slide and applied to one end of a second slide which should lie on firm support. When the blood has spread along the edge of the smearer it should be slowly and firmly drawn over the surface of the receiving slide. The drop should, if possible, be small enough to be exhausted in the smearing, and the thickness of the layer can be fully controlled by the degree of pressure. The blood should be pushed before the smearer and not trailed after. (See Fig. 21.)

Many prefer to use cover-glasses in spreading the blood. One polished cover-glass is touched to the drop of blood and applied to a second cover, all corners projecting. When the blood has spread to the edges the cover-glasses are gently slid apart without pressure. The cover-glasses should be handled with forceps, otherwise the moisture of the finger will often crenate many cells.

The writer prefers to use glass slides, finding that beginners are much more successful with the slides than with cover-glasses, that, after very little practice, every specimen can be spread successfully; that forceps are not required; that slides may be handled and transported without fear of breakage; that they need not be mounted, and, therefore, do not fade like cover-glass specimens, which require mounting in balsam; that they may be restrained if necessary; and, above all, that they may safely be *fixed* in the free flame.

FIG. 21.



Method of making blood smears.

After spreading, all specimens should be well dried in the air. They may then be kept for weeks if wrapped in tissue paper and kept from moisture, but it is better to *fix* them at once.

Fixation. 1. **Heat.** In routine work one may discard all other methods for that of *fixation in the free flame of a Bunsen burner.* The slide, specimen side up, is passed slowly through the flame until it is decidedly too hot for the hand to bear. At this temperature, which probably varies between 110° and 150° C., fixation is complete in one to two minutes.

A little practice will give the confidence necessary to heat the slides hot enough, as one's initial failures from this method almost always result from incomplete fixation and subsequent vacuolization of the red cells. Overheated slides can usually be seen to change color in the flame, after which the red cells stain yellowish with eosin. The beginner is strongly recommended to perfect himself in this simple method of fixation.

Small ovens provided with a thermometer are made for the fixation of blood slides, and may be used when many specimens are in hand, or when one does not care to risk the free flame. Specimens should be exposed five to ten minutes to a temperature of 110° to 120° C.

2. **Alcohol.** Fixation for ten to thirty minutes in 97 per cent. alcohol, or in equal parts of alcohol and ether, is a very reliabe method in very general use. Specimens may be left in alcohol for twenty-four hours, but do not then stain quite so well. There appears to be no advantage in adding ether to the alcohol, which even without mixture with the more volatile agent must frequently be replaced. Methyl alcohol fixes much more rapidly than ethyl, requiring only one to two minutes. It may be advantageously employed as a routine fixative, and it is used as a combined fixative of blood and solvent of dyes in Jenner's and Goldhorn's stains. Fixation in alcohol is to be specially recommended for the malarial parasite, but is unsatisfactory when Ehrlich's triacid stain is to be used.

3. **Fixation by Vapors.** Specimens may be fixed by being laid, specimen side down, over a wide-mouthed bottle containing 25 per cent. formalin, to which the exposure is five minutes, or 2 per cent. osmic acid, to which expose two minutes. Both these fluids have to be replaced frequently, they considerably alter the staining relations of the blood cells, and are inferior to other methods of fixation.

4. **Fixation without Drying.** Jolly and others claim that fixation after drying destroys many of the essential characters of leucocytes, which may be demonstrated in specimens fixed while moist in solutions of chromic acid. Flemming's stronger solution gave the best results in Jolly's hands (1 per cent. chromic acid, 15 parts; 2 per cent. osmic acid, 4 parts; glacial acetic acid, 1 part). Other fixatives recommended for the same purpose are saturated bichloride in 0.6 per cent. salt solution (Hermann's fluid). All these fixatives undoubtedly give better demonstration of nuclear structures and mitotic figures than can be obtained after fixation with drying.

Methods of Staining Dry Blood Specimens. 1. **Eosin and Methylene Blue.** The solutions required are: A saturated alcoholic solution of Ehrlich's blood-eosin. A saturated watery solution (1 per cent.) of Ehrlich's rectified methylene blue. The latter should be at least one week old, as fresh solutions lack selective quality and stain the specimen diffusely. After several weeks methylene blue in solution diminishes in staining power, while the alcoholic eosin absorbs water, and becomes less selective and more powerful.

In staining flood the specimen with eosin for a few seconds and wash in water. If the stain is not effective add more eosin, but the water on the slide dilutes the alcohol and renders the second application of eosin much more powerful than the first. Next flood the specimen repeatedly for one minute with methylene blue, wash hastily in water, and dry.

This method may be recommended for all ordinary examinations. The blood is stained as shown in Plate II., readily distinguishing the various forms of normal leucocytes. It does not stain neutrophile granules in leucocytes unless the action of eosin has been prolonged, in which case the neutrophile leucocytes can be distinguished from the eosinophile only by the size of the granules. Its chief advantage is the clear differentiation of basophilic leucocytes and of nuclear structures. It clearly demonstrates the malarial parasite, but in this field is greatly inferior to Nocht's method. Its chief disadvantage is the danger of overstaining with eosin, which prevents the full action of methylene blue.

2. **Ehrlich's Triacid Mixture.** This fluid has the following composition :

Saturated watery solution of orange G	120-135 c.c.
" " " acid fuchsine	80-165 "
" " " methyl green	125 "

To these add :

Aqua	300 "
Absolute alcohol	200 "
Glycerin	100 "

The attempt to prepare this mixture is not always successful. The smaller quantities of orange G and acid fuchsine are best employed, and the solution of methyl green, well seasoned, should be added slowly, with stirring, to the mixture of the other dyes. The water should be added next, then the alcohol, and, finally, the glycerin, with constant stirring. After standing one week the mixture is ready for use. Grübler's preparation of this mixture is in the market and is reliable.

In staining it is only necessary to flood the specimen with the dye for one to two minutes, and wash hastily in water. It cannot overstain, but overheated specimens are usually faint, and the red cells are yellowish. It stains neutrophile and eosinophile granules deep red, the latter being distinguished by their size. It is, therefore, indispensable in the diagnosis of leukemia. It is a poor nuclear stain, fails to demonstrate the structure of normal mononuclear leucocytes, and does not stain the malarial parasite.

On account of the uniformity of its results many prefer it to eosin and methylene blue as a routine method.

3. **Jenner's Stain.** Jenner's method of fixing and staining blood has now withstood sufficient trial to warrant its acceptance as one of the most important recent methods in blood technics. The specimens are fixed and stained in the same solution, which is prepared as follows: equal parts of 1.2 per cent. to 1.25 per cent. of watery solution of Grübler's yellow water-soluble eosin and of 1 per cent. watery solution of Grübler's medicinal methylene blue are mixed together in an open basin, thoroughly stirred, and allowed to stand twenty-four hours. The mixture is then filtered, dried in the air, or oven, at 55° C., the filtrate powdered, shaken up with distilled water, and washed on a second filter. It is again dried, powdered, and stored in bottles for use. The stain is prepared by dissolving 0.5

gramme of the powder in 100 c.c. pure methyl alcohol (Merck's "for analytical purposes").

Very thin smears of blood, made on thoroughly clean slides, are dried in the air. The dye is poured on the specimen, and staining is complete in one to three minutes. The specimens are washed, preferably in distilled water, until of a pink color, which usually appears in ten seconds. All the cells, their nuclei, and the various granules, are well differentiated, while the malarial parasite is densely stained and only in the larger parasites does the chromatin fail to appear deeply red stained. For this last purpose the method is inferior to Nocht's. The powder or fluid dye may be obtained from New York dealers.

4. **Demonstration of "Mast-cells."** The large basophilic granules of these cells retain basic dyes with tenacity, and may be demonstrated by a mixture of one of these dyes, with a strong decolorizer, which removes the stain from most other basophilic structures. Ehrlich's dahlia solution is adapted to this purpose:

Absolute alcohol	50.0 c.c.
Glacial acetic acid	12.5 "
Distilled water	100.0 "
Add dahlia to saturation.	

Stain several hours, wash in water, decolorize in alcohol or more rapidly in 20 per cent. Ac., until the nuclei fade, and wash in water. The nuclei of leucocytes are then very pale blue, the mast-cell granules very dark blue or black.

Recent experience has shown that mast-cell granules are very soluble in water and that their reliable demonstration requires fixation and staining by alcoholic solutions.

Michaelis recommends a solution of thionin or kresyl violet in 50 per cent. alcohol. The author prefers to use Goldhorn's solution in strong methyl alcohol of eosin and polychrome methylene blue.

ESTIMATION OF THE SPECIFIC GRAVITY OF THE BLOOD.

Hammerschlag's method is the most practical of the various indirect procedures devised for this purpose. A small urinometer of suitable dimensions is partly filled with a mixture of chloroform (s. g. 1526) and benzine (s. g. 0.889) of a gravity of about 1060. By means of a pipette, such as the red-cell mixer of Thoma, a drop of blood, expressed with the usual precautions, is transferred to the fluid. In expelling the blood the tip of the pipette should be submerged and no air should be allowed to pass out with the blood. The drop should not be very minute in size and should float on the fluid. If it is allowed to sink it will often be lost by spreading out on the bottom of the vessel. By adding chloroform or benzine drop by drop, as required, and carefully mixing by inverting the urinometer closed by the palm, a mixture is secured in which the drop neither rises nor sinks, but which is of exactly the same density as the blood. The specific gravity of the mixture and of the blood may then be taken as with urine. The urinometer should be graduated up to 1065, and should be tested in distilled water at 60° F. The apparatus should be clean and perfectly dry. The mixture may be filtered and used repeatedly.

Great accuracy can hardly be expected from this method, yet it is sufficiently reliable for clinical purposes. Errors arise from changes in the blood drop during and after its transfer to the mixture, from evaporation, from the escape of gases, from the inclosure or adherence of minute air bubbles, and perhaps also from the possible action of chloroform and benzine upon the blood, which is at present an unknown factor. Accordingly, Hammerschlag's method gives a uniformly higher gravity than is obtained by more accurate direct estimates. Practically, in performing the test one finds that the behavior of the drops varies, for reasons which are not clear, some rising and others falling in the same mixture. A large drop should be secured and followed in the test.

Since the specific gravity of the blood in simple anemia varies principally with the Hb, the percentage of Hb may in many cases be calculated with considerable accuracy from the specific gravity. Hammerschlag has prepared the following table showing the relation of Hb to the specific gravity as determined by his method:

Specific gravity.	Hb.
1033–1035	25–30 per cent.
1035–1038	30–35 "
1038–1040	35–40 "
1040–1045	40–45 "
1045–1048	45–55 "
1048–1050	55–60 "
1050–1053	65–70 "
1053–1055	70–75 "
1055–1057	75–85 "
1057–1060	85–95 "

The suggestion that Fleischl's hemoglobinometer be discarded for this indirect method of estimating Hb has not found favor. The changes in the plasma in severe anemia, leukemia, dropsy, and diarrheal diseases, render this practice always unscientific and frequently very unreliable (cf. Siegel, and Stintzing and Gumprecht).

FIG. 22.

Capillary glass tube adapted to various details of blood analysis.

Schmaltz's Method. A thin-walled capillary glass tube is prepared, about 12 mm. in length, and of a calibre of about ⅔ mm. at the ends and 1½ mm. at the middle. This is thoroughly cleaned, dried, and weighed before and after filling with distilled water. After drying with ether the tube is filled with blood and again weighed. The weight of the blood divided by that of the water gives the specific gravity of the former.

This is the best of the methods requiring a small quantity of blood, and in experienced hands gives more accurate results than

Hammerschlag's method. It is to be especially recommended in experimental and laboratory work, but usually requires too much blood for its adoption as a routine clinical method, although the quantity required, two drops, is not great. The chief sources of error are in the difficulty of thoroughly cleaning the tube and inaccuracy in the use of the scales. Schmaltz found by controlling his results by the use of salt solutions of known density that the error did not exceed 0.003 in the computed gravities.

Comparing the percentage of Hb obtained by Fleischl's method with the specific gravity obtained by his own method, Schmaltz constructed the following table:

Specific gravity.	Hb.	Specific gravity.	Hb.
1030	20 per cent. ±	1049	60 per cent.
1035	30 "	1051	65 "
1038	35 "	1052	70 "
1041	40 "	1053.5	75 "
1042.5	45 "	1056	80 "
1045.5	50 "	1057.5	90 "
1048	55 "	1059	100 "

ESTIMATION OF THE ALKALESCENCE OF THE BLOOD.

The determination of the alkalescence of the blood is attended with very great practical difficulties. This alkalescence being referable to the presence of carbonates, bicarbonates, and of albumins which are retained in solution by acid phosphates, it is always difficult to judge of the changes in these principles and the consequent variations in reaction produced by the procedures required in alkalimetry. If serum alone is titrated, the alkaline principles of the clot are left out of account, and if "laked" blood is employed there is an uncertain factor in the chemical changes produced, especially in the delicately balanced albumins and phosphates, during the solution of red cells.

Nevertheless it appears from a considerable number of painstaking studies by Landois, v. Jaksch, Kraus, Tausczk, Lowy, Schultz-Schultzenstein, Limbeck, and Rigler, that in blood and in serum there is a fairly constant group of alkaline principles which may be rather accurately measured and which have a distinct and important relation to disease (cf. Blood in Fever).

Four of the many methods employed may be recommended as most reliable.

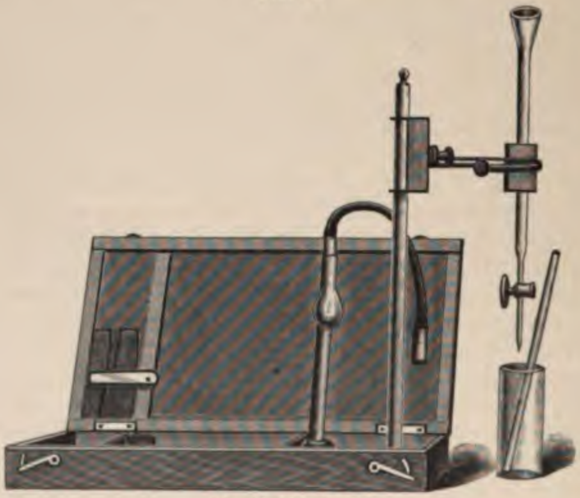
1. **Lowy's Method.** In a 50 c.c. flask containing 45 c.c. of 2 per cent. solution of ammonium oxalate, 5 c.c. or less of fresh blood are accurately measured and dissolved.

Of the solution 5 c.c. are titrated by means of a $\frac{1}{25}$ normal solution of tartaric acid, using litmus paper as an indicator. The latter may be prepared by soaking prepared paper in an alcoholic solution of litmus to which dilute HCl has been added until a violet color appears. The end reaction is obtained by adding a drop of blood solution to the paper and closely inspecting the color of the outer zone into which the fluid diffuses. The result is not affected by changes in temperatures.

Engel's Alkalimeter. Engel has devised an apparatus for the clinical estimation of alkalinity according to Lowy's method. A large drop of blood is drawn into a special pipette up to the mark 0.05 and diluted with distilled water to the mark 5.0. After shaking the dissolved blood is discharged into a glass cylinder and titrated by $\frac{1}{75}$ normal solution of tartaric acid (ac. tartar., 1 gr.; aq. dest., 1 litre). The acid is added drop by drop until a distinct red zone appears, when a drop of blood is allowed to diffuse through litmus paper. In normal blood about ten drops of acid bring the end reaction.

COMPUTATION. If 0.4 c.c. of acid is required to neutralize 0.05 c.c. of blood, 8 c.c. of acid will be required to neutralize 1 c.c. of blood. One cubic centimetre of $\frac{1}{75}$ normal tartaric acid neutralizes 0.533 mg. NaOH (Engel), so that 8 c.c. of acid solution indicate the presence in 1 c.c. of blood of 4.264 mg. of NaOH, to which terms the alkalinity of the blood is usually reduced.

FIG. 23.



Engel's alkalimeter.

2. **Method of Schultz-Schultzenstein.** By means of the pipette of the Fleischl hemoglobinometer, 5 or 7.5 mg. of blood are measured, which is dissolved in 12 c.c. of distilled water. This is acidified by adding 1.5 c.c. $\frac{1}{100}$ normal H_2SO_4. After careful mixing a drop or two of ethereal solution of erythrosin is added as an indicator and the solution titrated, with frequent stirring, by $\frac{1}{600}$ normal solution of KOH. The end reaction is shown by the first appearance of a red color in the supernatant ether. The test must be performed speedily to avoid obscuring the end reaction by a layer of fibrin precipitated by the ether.

The requirement of a very small quantity of blood renders this method specially suitable for clinical purposes.

3. **v. Limbeck's Method.** To about 200 c.c. of boiled distilled water are added 5 c.c. decinormal HCl solution, and drop by drop, with stirring, 5 c.c. of *serum* spontaneously expressed from a clot. The stirring rod should be covered with black gutta-percha. The resulting clear and slightly opalescent fluid is now titrated with decinormal solution of NaOH. After adding a few drops a precipitate forms which soon dissolves. The end reaction is reached when the abundant precipitate (of albumin) no longer dissolves, which is best determined by finding a flocculent precipitate persisting on the black rod.

The same quantity (5 c.c.) of fresh blood may be titrated by this method and the total alkalinity of the blood determined. In adding blood, however, it must be dropped very carefully into the hot water to avoid coagulation.

The chief technical difficulty with this method lies in detecting the end reaction. In case of doubt the precipitate may be dissolved by adding 1 to 2 c.c. of acid, as above, and titrating as before.

There is also the objection that it takes no account of the alkalinity referable to albumins. Limbeck doubts, however, if the capacity of albumins to neutralize acids ever comes into action in the body, and argues that his method estimates exactly, without regard to the quantity of albumin, the alkalinity of the salts present in the blood.

4. **Rigler's Method.** The blood flows from the vein through a canula into two vessels: (1) 3 to 4 c.c. in a tube for centrifuging; (2) a similar quantity into a 50 c.c. flask containing 10 c.c. of 96 per cent. alcohol.

The serum is obtained by centrifuging, and is transferred to a flask containing 10 c.c. of 96 per cent. alcohol.

Both flasks are weighed both before and after the addition of blood or serum to determine the weight of blood.

After shaking the specimens stand for half an hour, and then to each is added 10 c.c. of neutral distilled water. The alkalinity is determined by titration with $\frac{1}{50}$ normal H_2SO_4, using red and blue litmus paper as indicators.

5. **Wright** has devised and extensively used one of the most practical clinical methods of estimating the alkalinity of the blood, for the full explanation of which the reader should consult the original description.

Determination of the Coagulability of the Blood.

Vierordt and Wright have employed methods of determining the coagulability of the blood the results from which do not appear to be sufficiently accurate even for clinical purposes (Limbeck).

Estimation of the Osmotic Tension of the Plasma.

Although comparatively little attention is usually paid to the osmotic tension of the plasma, it is evident from the frequent occurrence of hemoglobinemia in various forms of severe anemia, malarial and especially hemoglobinuric fever, jaundice, acute poisonings, etc., that the condition of the blood in this respect is of prime importance in the clinical and pathological study of these diseases. In *Hamburger's method* we possess ready and very exact means of investigating this problem.

In a series of test tubes are poured small quantities, accurately measured, of the serum to be examined, and to each is added an increasing quantity of distilled water. The surface of the mixed fluids in each tube is then touched with a pipette holding a very little normal blood, and the tubes are allowed to stand twelve hours. By that time the cells have settled to the bottom in some tubes, while in others the supernatant fluid is tinged with dissolved Hb, and one notes that tube in which the first traces of Hb appear. Normal red blood cells begin to lose their Hb in solutions of salt containing any less than 0.46 per cent. of NaCl. Estimated in terms of NaCl that tube of the series which shows the first faint traces of dissolved Hb, therefore shows the same osmotic tension as a 0.46 per cent. solution of salt. The dilution being known the tension of the original serum can be computed as follows: Suppose that to 1 c.c. of serum 0.9 c.c. of water added, caused the solution of Hb. Then the tension of the original serum is equivalent to $1 + 0.9 \times 0.46 = 0.874$ per cent. of NaCl.

Hamburger[4] points out that all red cells do not dissolve with equal rapidity in the same salt solution, and he defines as *minimum resistance* that strength of salt solution in which the weakest cells begin to dissolve, and as *maximum resistance* that solution in which the strongest cells begin to dissolve. In order to obtain comparable results he recommends for universal use a series of simple tubes in which is secured a dilution of blood in proportion of 1 : 40 of salt solution. He determines the minimum resistance by adding 0.05 c.c. of blood to each of several salt solutions, allows the specimens to stand for two hours, and centrifuges in order to determine the first appearance of dissolved Hb. The apparatus with twelve tubes can be obtained from W. J. Weller, Kerk Stratt, Utrecht.

CRIOSCOPY.

In 1898 Koranyi pointed out that certain important clinical deductions could be drawn from the determination of the freezing point of the blood and urine. The method is based upon the fact that the presence of elements in solution in a fluid increases the osmotic tension of the fluid and lowers its freezing point, both effects following invariable physical laws.

The freezing point of normal blood he found to be 0.56° lower than that of water—a fact which is commonly indicated by the formula $\Delta = 0.56°$. A series of clinical studies showed that in uncompensated heart disease the lowering of the freezing point of the blood, Δ, is increased, reaching as much as 0.67°, although the chlorides of the blood are diminished. This result he shows is owing to the retention of CO_2 in the system.

In nephritis Δ was shown to be much increased, reaching 0.71° in a case of uremia. Various characteristic changes were noted in the blood in anemia and in fevers, where in the absence of cyanosis Δ was less than 0.56°, and in hemoglobinemia, in which Δ rose to 0.70°. Various contributions regarding the clinical value of the test have appeared. Koeppe noted variations in healthy subjects between 0.558° and 0.570°, and in hysteria, menstruation, gastritis, cancer of pylorus, diabetes, nephritis, neurasthenia, pleurisy and pneumonia, variations between 0.508° and 0.634°.

Lindemann finds that in disease of the kidneys the freezing point of the serum is unaffected as long as uremic symptoms are absent, while in uremia Δ rises to 0.70°.

Kummel, in ten cases of nephritis, cystic kidney, and anuria after operation, always found Δ increased to 0.60° to 0.65°, and once to 0.71°. Ogston reports various conditions in which the value of the test appears very uncertain. Ceconi and Micheli could not distinguish by the method the stage of an inflammatory exudate, nor between exudates and transudates, but found that the freezing point of the cerebrospinal fluid was not higher than normal (0.56°), while in uremic seizures simulating meningitis Δ was increased.

In pneumonia Δ was always increased to 0.60°, while in typhoid fever several observers have demonstrated no change. Carrara finds crioscopy of the blood serum the most reliable test for the medico-legal diagnosis of death by submersion. The absorption of fresh water through the lungs, gastro-intestinal tract, and skin, raises the freezing point of blood serum, while salt-water lowers it, in each instance more markedly in the left than in the right heart. His results were as follows:

Dog, drowned in fresh water, serum of right heart . . .	$\Delta = 0.42°$
" " " " " left heart . . .	$\Delta = 0.29$
Man, drowned in sea water, serum of right heart . . .	$\Delta = 1.04$
" " " " " left heart . . .	$\Delta = 1.18$

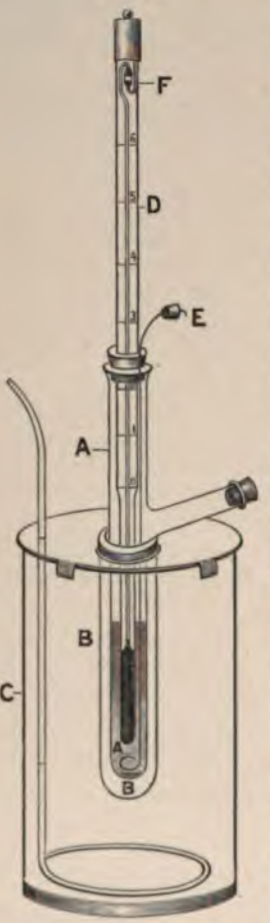
In the determination of the freezing point of blood and blood serum, Beckmann's apparatus is employed, and preferably the modification of Lindemann.

About 10 c.c. of blood is required, and may be drawn from the basilic vein. Coagulation makes no difference in the freezing point (Ogston).

Beckmann's Apparatus. Description of Apparatus (Fig. 24). The large vessel (C) for holding the mixture of salt and ice is fitted with a perforated iron lid through which passes the handle of a wire stirrer, while in the centre is an opening for the tube (B). Another tube (A) containing the liquid to be tested, the thermometer (D), and a small wire or glass stirrer (E) fits in the mouth of the large tube.

The thermometer is of peculiar construction, graduated in $\frac{1}{100}$° C., and permitting very accurate readings over a scale of 10°. It contains the usual main reservoir at the bulb and also an accessory reservoir at the top of the column (F), by means of which one may add to or take from the column of mercury at will. A thermometer with "fixed zero" and without an accessory reservoir may be obtained from the makers of Beckmann's apparatus as modified by Lindemann, but even with this thermometer the exact freezing point of water on the instrument varying from day to day must first be determined as described later. Lindemann's apparatus has the advantage of requiring not more than 5 c.c. of serum.

Fig. 24.



Beckmann's apparatus.

Application of the Test. The large jar is half filled with finely crushed ice and salt. The thermometer must first be "set" so that the top of the column of mercury at freezing point falls about the middle of the graduated scale. This may be done by immersing the bulb in warm water until the mercury in the column joins that in the accessory reservoir. Then fill the inner tube with distilled water and cool to about 10° C., immersing an ordinary thermometer in the water. Now place the thermometer of the instrument in the cool water, and when it is thoroughly cool break the column of mercury in the reservoir by tapping it sharply with the finger.

The distilled water is now frozen with the thermometer immersed, and the exact point to which the broken column of the mercury falls is taken as the zero of the instrument. It will be found that the moment that the water congeals the mercury falls slightly below the true freezing point, shortly to rise and remain steady at the exact point.

With Lindemann's thermometer the breakage of the column is not required, but the exact freezing point of water on the thermometer must be determined. Having determined the zero of the instrument, the blood or serum to be tested is placed in the inner tube, cooled to near the freezing point, and the thermometer immersed in it. As the fluid cools it must be stirred by the glass stirrer. When the fluid congeals the mercury drops a fraction of a degree, but soon rises and becomes stationary at the freezing point of the fluid. The difference between the freezing point of water and that of the fluid, Δ, may then be determined by the reading, which should be made as soon as the column becomes stationary.

Bremer's Specific Reaction of Diabetic Blood.

Saturated watery solutions of eosin (watery?) and of methylene blue are mixed in about equal proportions so that a neutral reaction is obtained, and the mixture produces little or no stain on litmus paper. A precipitate forms, soluble in alcohol, insoluble in water, which is filtered, washed, dried, and powdered. To twenty-four parts of this powder are added six of powdered methylene blue and one of eosin Of this mixed reddish brown powder 0.025 to 0.05 grm. are dissolved in 10 c.c. of 33 per cent. alcohol, in which solution the specimen is stained for four minutes.

The specimens are prepared by smearing the blood on glass slides or covers, and fixed by boiling in equal parts of alcohol and ether for four minutes. This may be accomplished by placing the bottle of alcohol and ether in hot water at 60° C.

After washing the stained specimens in water, diabetic blood has a greenish tint, while normal blood is reddish violet, and on microscopic examination the erythrocytes of diabetic blood are found to be greenish, while those of normal blood are red.

Bremer found the reaction in 50 out of 51 cases of diabetes. The negative result was obtained in a well-established case, a boy of sixteen, in whom glycosuria began after an electric shock. He found that the reaction persisted in the absence of glycosuria; that normal blood floated on diabetic urine for fifteen minutes gave the reaction; that blood treated with solutions of glucose failed to give the reaction; that the blood in glycosuria artificially produced in animals by phloroglucin gave the reaction, while in that produced by phloridzin it did not.

The nature of the reaction is not understood. While some observers have convinced themselves that the presence of glucose is not sufficient to bring about the altered staining qualities of the blood, Hartwig concluded that the glucose first causes a change in the Hb which shows itself in the altered reaction of the red cells to aniline dyes. Other reducing agents besides glucose are probably of importance in this relation (R. Muller). Some have attempted to explain the reaction by reference to the changes in alkalescence of the blood. Hartwig found the reaction to disappear after the addition of alkali to the blood. Although Lowy found a pronounced reaction in cases with relatively high alkalescence, Schneider and others have shown that the reaction runs parallel with the quantity of abnormal acids in the blood.

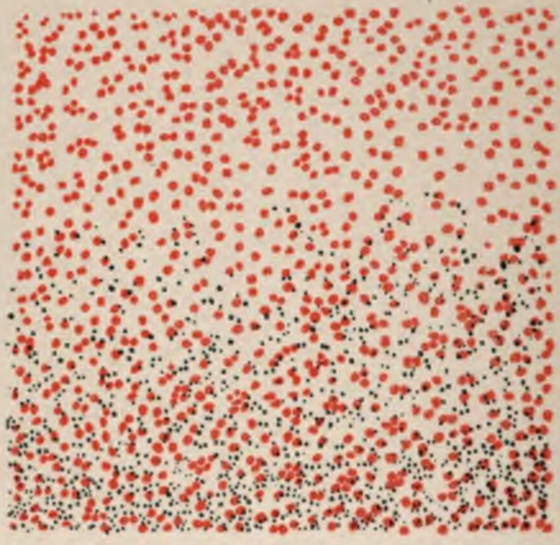
Various modifications of the above most approved method have been employed successfully by Bremer and others. One of these, employed by Bremer, is as follows: 1 per cent. solutions of Congo red or of methylene blue stain diabetic blood very slightly, while 1 per cent. solution of Biebrich scarlet stains it intensely. A directly opposite relation holds with normal blood. Rather thick smears of blood should be used with this procedure, and the colors compared by the naked eye.

The value of Bremer's test has been confirmed by Le Goff, Eichner and Folkel, Lepine and Lyonnet, James, Jeanselme, Badger, and Hartwig, but similar reactions have been found in normal blood in leukemia, Hodgkin's disease, exophthalmic goitre, and multiple néuritis. A partial reaction has been obtained in cachectic conditions, and Bremer failed to find it in a case of glycosuria of neurotic origin. Yet in most conditions, other than diabetes, the reaction has been found, when present, to be inconstant, and to occur in a very small proportion of cases (Lepine, Eichner, Hartwig).

The technical difficulties in carrying out the test are considerable, while a slight variation in technique appears to vitiate the result, as is indicated by the failures reported by Patella and Mori, after both Bremer's and Williamson's methods.

Williamson finds that diabetic blood decolorizes solutions of methylene blue, while normal blood does not. His test is performed as follows: 20 c.mm. (2 drops) of blood are dissolved in 40 c.mm. of water, and to the solution is added 1 c.c. of methylene blue (1 : 6000 solution) and 40 c.mm. of *liquor potassæ* (s. g. 1.058). The vessel is then placed in boiling water for four minutes; diabetic blood decolorizes the solution, normal blood leaves it deep blue. Diabetic urine has the same effect.

FIG. 25.



Pronounced lipemia. Specimen treated with osmic acid. Lower half shows extracellular fat globules, upper half having been cleared by oil of turpentine. (Gumprecht.)

Williamson found this reaction in 6 diabetics and failed to find it in 160 cases of other diseases, including one of leukemia.

Demonstration of Glycogen in Blood.

Gabritschewsky's method may be employed. The blood smears, thoroughly dried in the air, are stained for several minutes in: iodum pur., 1; KI, 3; aq., 100; acacia pulv. in excess.

The presence of glycogen is indicated by the appearance of mahogany-brown granules of variable size, in leucocytes and plasma. (See Plate XII.)

Czerny claimed that this method demonstrates the presence in the blood not of glycogen but of a carbohydrate more nearly related to amyloid. Huppert's later studies on the blood of animals support the belief that the substance thus demonstrated is really glycogen.

It is probable that many colorless globules visible in leucocytes treated by ordinary staining methods are referable to the former presence of glycogen, which is soluble in water and of which the reactions are largely destroyed by heat.

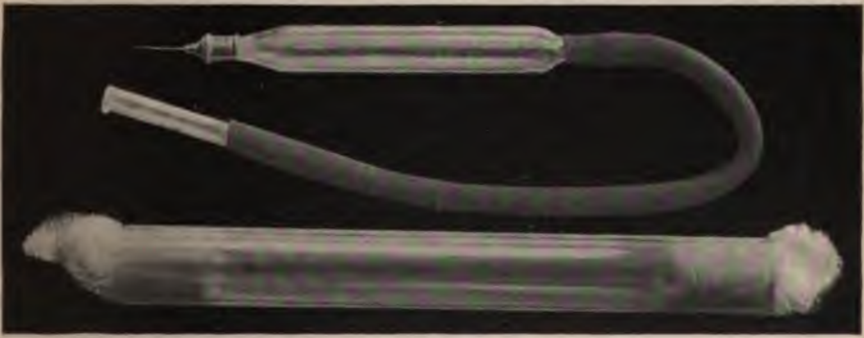
Demonstration of Fat in the Blood.

The blood smear is fixed in 1 per cent. osmic acid for twenty-four hours and counterstained with eosin. The fat particles are then stained black. Since all that blackens under osmic acid is not fat, a control preparation should be fixed twenty-four hours in alcohol and ether, then in 1 per cent. osmic acid for twenty-four hours, counterstained with eosin, and the extraction of the fat by ether demonstrated by the absence of black particles in cells and plasma.

BACTERIOLOGICAL EXAMINATION OF BLOOD.

Apparatus. Any aspirating syringe of the capacity of 10 c.c. which can be thoroughly sterilized may be used for drawing the blood, but the difficulty of sterilizing the ordinary syringe renders it advisable to employ a special apparatus without joints and permitting the adjustment of the needle on a ground-glass orifice (Fig. 26).

FIG. 26.



Blood aspirator. (Half size.)

The other end of the tube, considerably narrowed and bulbed, is plugged with cotton, and over it a rubber tube is slipped, through which the blood is sucked by the operator. The glass tube and needle are inclosed in a glass case, sterilized by heat, and kept ready for use. Such an apparatus—a modified form of one devised by Dr. James—has been made for the writer by Stohlmann, Pfarre & Co., New York City.

Operation. The blood should be drawn from the median basilic vein. The skin of the arm must be thoroughly washed with soap and water, cleaned with alcohol, and sterilized with bichloride solution 1 : 500, or formalin 5 per cent.

In most female subjects an Esmarch bandage must be applied at the axilla to render the basilic vein prominent, but in many male subjects this is unnecessary. It is better to incise the skin over the vein before inserting the needle, but this precaution is not essential under ordinary circumstances. When the needle enters the vein obliquely the blood should flow into the tube, and the flow may be hastened by suction through the rubber tube.

At least 10 c.c. should be drawn, a quantity which is usually of no consequence to the patient. The elbow may be snugly bandaged after the operation.

Preparation of Cultures. Before clotting the blood should be distributed in culture media. The medium chosen depends upon the particular organism which one expects to demonstrate. For the usual pathogenic species, staphylococcus, streptococcus, typhosus, etc., ordinary bouillon suffices, but for the gonococcus, ascitic broth should be preferred.

The dilution of the blood should be considerable, and for 10 c.c. of blood it is advisable to employ at least 300 c.c. of broth.

Unusual care should be employed at every step of the process to prevent contamination.

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CHAPTER II.

CHEMISTRY OF THE BLOOD.

THE CHEMICAL COMPOSITION OF RED BLOOD CELLS.

IN the analyses of Schmidt, Hoppe-Seyler, and Judell the red cells were separated from the serum by the addition of salts (Na_2SO_4, NaCl), a procedure which considerably alters the chemical composition of cells and plasma. The accuracy of their results, which are the best available, is therefore only approximate.

The specific gravity of red cells is usually about 1088. They contain about 90 per cent. of oxyhemoglobin and a small proportion of a globulin-like albumin (nucleoproteid) coagulating at 75° C. There are also traces of lecithin and cholesterin. The principal salts are phosphates of Na, K, Ca, and Mg, and chloride of K, whereas in the serum the chief salt is NaCl.

In cholera, dysentery, and dropsy, Schmidt found the red cells to be of increased specific gravity in proportion to the duration of the exudative process, while their chemical analysis showed that they participate in the changes which first affect the serum, losing first water, then salts, and finally albumin. More recently v. Jaksch,[1] after establishing the normal content in N of the red cells, followed the variations in this principle in various diseases. He found very marked and irregular variations both in health and disease, which indicate that the method used (determination of total N) was unreliable.

Biernacki, estimating the dry residue of red cells settled after the addition of sodium oxalate, found a *normal* residue of 29.28 to 30 per cent.; in carcinoma of the esophagus, 27.9 per cent.; in tabes with anemia, 25.51 per cent.; in chlorosis (Hb 25 per cent.), 22.24 per cent.; in chronic nephritis (Hb 20 per cent.), 22.88 per cent. Among the important observations of Biernacki's are the increased content in water of the red cells in hydremia, the general parallelism between P_2O_5, Fe, and K, and between water and NaCl in the red cells.

Hemoglobin (Hb) and its Derivatives.

Hemoglobin belongs to the group of proteids, containing about 96 per cent. of albumin and 4 per cent. of an iron-holding pigment, *hemochromogen*. Hb exists in the red cells in combination probably with the nucleoproteid of the stroma. The nature of this union is not certainly understood, but it renders Hb comparatively insoluble, greatly concentrated, and capable of actively forming unstable compounds with oxygen (Hoppe-Seyler, Gamgee).

Its chemical composition is very complex and apparently variable, but its spectroscopic relations are constant. In the circulation it exists principally as *reduced Hb* in the veins, and in molecular union with oxygen as *oxyhemoglobin*, in the arteries. One gramme of saturated oxyhemoglobin yields about 1.16 c.c. of oxygen, but in the circulation the degree of saturation with oxygen varies, and in health is never complete. Meyer and Biarnes found in the arterial blood of a dog 76 per cent. of saturation with oxygen; after a large hemorrhage it rose to 85 per cent. of saturation, while after inducing CO poisoning it rose to 90 per cent. Limbeck found 97 per cent. of saturation with oxygen in the blood of a dog poisoned with potassium chlorate. It thus appears that with a relative loss of functionating Hb the demands of the system may cause a more complete saturation of the blood with oxygen than exists in health.

Oxyhemoglobin is bright red in color, and forms with difficulty yellowish red rhombic crystals. These crystals are very soluble in water and in very dilute solutions of alkaline carbonates, but when treated with strong alcohol they are modified and become insoluble (parahemoglobin of Nencki). They are insoluble in ether, chloroform, benzol, or carbon disulphide. Oxyhemoglobin is absolutely non-diffusible (Gamgee).

Hemoglobin (reduced Hb) is dichroitic. In thick layers or in thin layers of concentrated solutions it presents a dark cherry-red color, while very dilute solutions exhibit a green tint (Gamgee). This dichroism is characteristic of the blood of simple asphyxia. Reduced Hb is more soluble, but less easily crystallizable than oxyhemoglobin.

Methemoglobin is brownish red in color, readily soluble in water, and crystallizes in brownish red needles, prisms, and hexagonal plates. It contains the same proportion of oxygen as oxyhemoglobin, but in much firmer and probably in chemical union. Methemoglobin is found in the blood chiefly in cases of poisoning by a variety of substances, the so-called "blood poisons." Tschirkoff claims to have found it in Addison's disease, and Ruyter has recognized a very similar but not identical coloring matter in a case of malignant edema.

Hemoglobinemia, a solution of Hb in the plasma, a normal condition in the blood of some vertebrates, is in man always pathological, and results from lessened resistance on the part of the red cells and from abnormal states of the plasma. The former condition is probably concerned in cases of paroxysmal hemoglobinemia and in the destruction of blood which follows general burns (Lichtheim, Murri, Chvostek, Silberman), while the latter condition exists in the hemoglobinemia of acute poisonings and in cases referable to increased globulicidal power of the serum.

Carbonic oxide Hb is a firm compound of Hb and CO, and imparts a bluish red or rose-red color to the blood. Its crystals are similar to those of oxyhemoglobin, but more bluish and less soluble. In cases of poisoning by the inhalation of fire-damp or illuminating gas, CO-Hb is formed in considerable proportion and the respiratory capacity of the blood is largely destroyed. CO-Hb persists in the blood for several days, in cases that recover, and for a much longer period in fatal cases.

Hematin, one of the advanced decomposition products of Hb, is a dark brown or blackish, non-crystalline solid, decomposed at 180° C. It is insoluble in water, dilute acids, alcohol, ether, and chloroform, but dissolves in acidified alcohol and ether, and readily in dilute alkalies. It is found in old blood transudates, in the feces where digestive fluids have acted upon blood, and in the urine in cases of arsenical poisoning. Crystals of hemin, or the hydrochlorate of hematin, are formed in Teichmann's test.

Hematoidin is a crystalline derivative of Hb, found in old blood extravasations. It crystallizes in orange-colored rhombic plates, is soluble in chloroform, ether, carbon disulphide, and ammonium sulphide, contains no iron, and gives no absorption bands, although absorbing most of the violet end of the spectrum. It is generally regarded as identical with bilirubin.

Hemosiderin is an amorphous iron-holding product of the decomposition of Hb, and is abundantly found in the viscera in disease accompanied by much destruction of blood. It is probable that when red cells are disintegrated hemosiderin is formed by the action of living cells upon Hb, while hematoidin originates apart from any cellular activities (Perls, Thoma, Ziegler). Hemosiderin is blackened by ammonium sulphide and turned blue by acidified potassium ferrocyanide.

Melanin is a yellowish brown or black pigment produced by the action of the malarial parasite upon Hb. It is insoluble in water, alcohol, ether, chloroform, carbon disulphide, and acids in moderate strength, but is destroyed by heat, and is soluble in strong alkalies and in ammonium sulphide. It fails to yield the microchemical reactions of iron, and probably contains no iron.

The term "melanin" is also loosely applied to a variety of black pigments occurring in the body, some of which contain iron but whose origin is uncertain.

From "antialbumid," formed by heating egg-albumin with 3 per cent. H_2SO_4 for ten hours at 100° C., and from hemipeptone, Chittenden and Albro produced black melanin by digesting these substances with 10 per cent. H_2SO_4 and boiling for seventy-nine hours. The change they believe to consist in a process of hydrolytic cleavage. Sulphur and fatty acids were thrown off in the process, and the melanin was found to contain C, H, N, and S.

THE CHEMICAL COMPOSITION OF LEUCOCYTES.

Attempts to determine the chemical composition of leucocytes have been made by analyses of leukemic blood, of pus, and of fresh lymphoid tissue. In all of these it cannot be doubted that abnormal products were constantly present, so that the chemical composition of normal leucocytes cannot be learned by such methods. Probably Lilienfeld's analyses[1] of lymphoid tissue furnish the most reliable data.

In cadaveric leukemic blood various fatty acids have been isolated, and lecithin, a normal constituent of blood, has been found in excess. Glycogen has been repeatedly isolated in this and other conditions.

In fresh pus, Miescher found five different forms of albumin, an abundance of lecithin, and cholesterin, phosphates of Na, K, Ca, Mg, Fe, and chloride of Na.

In analyses of the thymus gland in calves Kossel and Lilienfeld isolated various albumins, principally nucleins, lecithin, and cholesterin, besides about 3 per cent. of inorganic principles. In older cells they believe that secondary products develop in the form of glycogen, protagon, and fats.

The *nuclein of Kossel* is a combination of the organic nucleinic acid which contains phosphorus, with albumin, and in the leucocytes is found in combination with another albuminous body, *histon*, with which it forms nucleohiston. Under certain conditions Kossel and Lilienfeld believe that nucleinic acid becomes free in the cells, and exerts bactericidal action before and during phagocytosis.

The *nucleins* are bodies obtained from animal (or vegetable) cells after digestion with pepsin to which they are resistant. They are rather insoluble in water, alcohol, and ether, give the biuret and Millon's reactions, and acting as strong acids and uniting with bases, they may be identified from the basic albumins by their tinctorial qualities. Boiled with dilute acids they yield "nuclein bases" or xanthin bodies; or treated with alkali they yield albumin and nucleinic acid. They are rich in phosphorus and iron. Nucleinic acid is of variable composition, but that from the calf's thymus is a combination of a xanthin body with a complex phosphoric acid (Hammarsten).

Milroy and Malcolm have demonstrated some peculiar reactions of the granules of leucocytes. They found that weak watery solutions of sodium carbonate or of acids remove the pseudoeosinophile granules. Injections of nucleinic acid reduce the number of pseudoeosinophile and eosinophile granules and cause them to become basophile. This change they explain on the theory that the granules are composed of a nucleoproteid and an albumin, the latter being removed by the nucleinic acid. The change being most marked in the pseudoeosinophile granules, they conclude that in these granules the albumin is less firmly bound than in eosinophile. The complete solution of the basophile residue of the granules seemed to be accompanied by an increase of excretion of P_2O_5.

THE CHEMICAL COMPOSITION OF BLOOD PLATES.

Since the true nature of blood plates has been at least partly shown the chief inquiry concerning them has related to their chemical composition. Lowit has strongly maintained that they are composed of globulin, a claim which is probably true of some of the bodies which appear in coagulating shed blood and which must be classed with the blood plates of different origins.

Lilienfeld[2] regards the substance of blood plates as belonging to the nucleo-albumins, and identifies them with the remnants of the nuclei of leucocytes, basing this opinion on their content in phosphorus, their resistance to digestion by pepsin, and their microchemical reactions. These observations may be regarded as definitely settling the question of their chemical composition, but do not prove their exclusive origin from leucocytes.

The Serum.

The Albumins of the Serum. Blood serum contains two albuminous bodies, serum albumin and serum globulin, which together form 7.62 per cent. (Hammarsten) or 8.26 per cent. (Schmidt) of the weight of normal serum.

The quantitative changes in these principles in disease are not usually very marked, owing to their relatively slight diffusibility.

From the studies of Becquerel and Rodier, Schmidt, v. Jaksch, Limbeck and Pick, it has been shown that the albumins of the serum are considerably diminished in severe anemias, and in nephritis or endocarditis with dropsy, but that in most severe infectious diseases they are but slightly reduced and bear a fairly constant relation to the other solids of the serum. If temporarily reduced by some exudative lesion, as in diarrhea, dysentery, etc., they are soon replaced if the disease continues.

Becquerel and Rodier, however, found the albumins much reduced in puerperal septicemia, and Schmidt found a marked increase in the concentrated blood of cholera. v. Jaksch,[2] determining the total N of the serum, found slight variations in many acute and chronic diseases, but a well-marked loss in leukemia, pernicious and secondary anemia, and chlorosis. Limbeck, using the same method, found less marked changes in cases of anemia.

The ratio of serum albumin to serum globulin, on the other hand, varies much more than does the total quantity of albumin in the serum. The normal limits are placed by Limbeck and Pick, for globulin, 16.9 to 38.3 per cent., for albumin 61.7 to 83.1 per cent. of the total albumin of the serum. When such wide limits may be found in normal subjects it is difficult to attach much importance to very considerable changes demonstrated in disease, as reported by Estelle, Hoffman, Halliburton, and Mya and Viglezio. In a considerable series of observations in various diseases Limbeck and Pick succeeded in showing only that serum globulin, the less diffusible principle, is less subject to change than is serum albumin, but they were unable to establish any other general rules.

The **inorganic salts** of the serum include phosphates, chlorides, carbonates, and sulphates, but in what proportion or form the alkalies and earths are combined with these acids is not definitely known. Sodium and potassium are combined especially as neutral chlorides, partly as phosphates and carbonates. Pathological variations in the phosphates of the serum are of slight degree and importance, so far as is known, probably on account of their occurrence in scant quantity and their relative lack of diffusibility. A retention of phosphates in the blood of pathological grade has not been demonstrated. The variations in the chlorides of the serum investigated by Schmidt, Biernacki, and Limbeck have not been found extreme, nor of notable pathological import, although this principle is chiefly responsible for the isotonic relations of cells and serum. A high percentage of chlorides is usual in anemias.

The **specific gravity** of the serum in disease varies slightly from the normal limits, 1025 to 1030 (Hammerschlag, Limbeck). While Becquerel and Rodier and Hammerschlag found the specific gravity of the serum normal in chlorosis but reduced in secondary anemia, Limbeck found it low in both (1021 to 1023). In infectious diseases Becquerel and Rodier found the serum of normal gravity.

The **color** of human serum is yellowish with a slight greenish fluorescence, but after a hearty meal the increased quantity of fat may yield a cloudy or whitish color. The coloring matter of normal human serum belongs to the group of luteins or lipochromes (Hammarsten). It is extracted by alcohol and by ether, yields a blue color with iodine and sulphuric acid, and on spectroscopic analysis causes an absorption of the violet and part of the blue, which is unaffected by reducing or oxidizing agents. Icteric serum causes more complete absorption, beginning sharply in the blue (Limbeck).

When, from lessened resistance of the red cells or abnormal conditions of the plasma, red cells are dissolved the serum contains Hb in solution (hemoglobinemia), as occurs principally in malaria, paroxysmal hemoglobinuria, septicemia, and with the blood poisons. In jaundice the serum has a characteristic orange-yellow color and bilirubin is readily detected.

Hemolytic Properties of Human Blood Serum. Maragliano (1892) first investigated the hemolytic power of pathological blood serum upon the red cells of human subjects (isolysis), finding that the serum in many cases of primary anemia, leukemia, purpura, pneumonia, malaria, typhoid fever, cirrhosis, etc., possessed in varying degree the power of dissolving healthy red cells. In the solution he obtained the spectrum of hematoidin. Heating to 50° to 53° C. did not destroy this hemolytic property. Various later writers have confirmed and extended these observations, and suggested various explanations of the toxic effects of blood serum, but the systematic study of the subject has been possible only since the contributions of Bordet and Ehrlich on the nature of hemolysis. Many of the earlier studies probably deal with other forms of hemolysis than those discussed by Bordet and Ehrlich.

Halpern (1902) reviews the subject to date, finding considerable but rather inconstant variations in the hemolytic power of blood serum in disease, both upon human and upon rabbit cells. In typhoid fever hemolysis was usually increased, and in septicemia it was decreased; but in most other conditions it varied slightly from that of normal blood. (See section on Hemolysis.)

THE WHOLE BLOOD.

Albumins. The albuminous principles of normal circulating blood include hemoglobin, serum albumin, serum globulin, and fibrinogen. In shed blood a nucleoproteid exists which is probably derived from the nuclei of leucocytes, probably also from red cells, which combines with calcium salts to form the fibrin ferment, and which is called "prothrombin." Traces of albumose have been found in the blood in various diseases, and other nitrogenous principles, occasionally present and commonly reckoned with albumins, are urea, uric acid, and xanthin bodies. Varying considerably with the method of determination, the proportion in which these elements exist in the blood has been placed for the entire group by v. Jaksch[2] at 22.62 per cent.; by Limbeck at about 25 per cent.; by Schmidt at 10.82 to 16.63 per cent.

A relative increase of total albumin is seen, according to v. Jaksch, in diseases accompanied by marked loss of fluids, as in cholera and severe diarrhea, but an absolute increase has not been demonstrated. In infectious diseases the albumins are moderately reduced, even when the number of red cells remains normal (typhoid fever). In nephritis there is usually but not always a reduction. In endocarditis there is little change. In chlorosis, pernicious anemia, and leukemia the total albumins of the blood are constantly diminished, but in secondary anemia the diminution usually noted sometimes fails. v. Jaksch found his lowest proportion, 8.46 per cent., in a case of gastric cancer.

Peptone has been demonstrated in the blood of leukemia by Ludwig, v. Jaksch,[3] and Freund and Obermayer, and *deutero-albumose* by Matthes. These observations were made on blood from the cadaver. Devoto and Wagner, who examined blood obtained during life, both failed to find peptone. The suspicion that the peptone previously demonstrated was of postmortem formation was followed up by v. Limbeck in a case of myelogenous leukemia. In the fresh blood no peptone was found, but in a specimen that had stood at room temperature for forty-eight hours albumose was demonstrated, but its exact character was not determined. The patient died three weeks later and *deutero-albumose* was demonstrated in the blood of the cadaver. v. Jaksch states that peptone (albumose?) is found in leukemic blood only when eosinophile cells are abundant. Freund's observation that peptone is found in the blood in cases of sarcoma but not in cases of carcinoma has not yet been confirmed.

The addition of small quantities of peptone to plasma *in vitro* reduces its content of CO_2 and its coagulability, and it seems probable that if peptone (or albumose) exists in the circulating blood a similar influence is exerted, *intra vitam*, by its presence.

Quantitative estimation of total albumin of the blood may be accomplished by one of two methods.

1. By precipitating the albumins by excess of alcohol, and weighing the dried precipitate.

2. By estimation of total N by Kjeldahl's method and multiplying the result by 6.25 (v. Jaksch).

By the first method an uncertain quantity of inorganic matter is carried down which cannot be thoroughly removed by washing, and which therefore disturbs the result.

In the second method N is derived from other principles besides albumins, and, as Limbeck and Pick have shown, there may be a difference of 10 to 20 per cent. in the results of the two methods.

Most of the nitrogenous principles other than albumins are soluble in alcohol, and some of the sources of error in this method are therefore removed by estimating the total N of the alcoholic precipitate. v. Jaksch has determined that by multiplying the percentage of N by 6.25 the average percentage of weight of albumin is obtained. Limbeck and Pick, after investigating the ratio between N and the weight of albumin from which it was derived, concluded that v. Jaksch's figure is sufficiently reliable in the great majority of cases, but that considerable inaccuracies may result in the cases of venous stasis, nephritis, and especially uremia, in which nitrogenous principles in the blood other than albumins are increased.

The determination of total N by Kjeldahl's method is generally employed in estimating the albumins of the blood. The method may be found fully described by Halliburton, *Chem. Phys. and Path.*, and Sutton, *Volumetric Analysis.*

Inorganic Principles of the Blood.

The Blood Ash. After incineration of two specimens of normal blood Schmidt found 0.84 per cent. and 0.91 per cent. of ash. This quantity was increased to 1 per cent. in a case of cholera, but the relation of ash to dry residue remained normal, 3.1 to 3.5 per cent. In the watery blood of nephritis, however, the ash was relatively much increased (6.5 per cent.).

Chemical analysis of the blood ash in health and disease have shown that pathological importance attaches principally to variations in the chlorides, phosphates, and the iron of the blood.

With regard to *the chlorides* (principally NaCl) the law has been established *that the larger the proportion of plasma the greater is the percentage of chlorides in the blood.* In pneumonia the chlorides are low, probably owing to diminished ingestion and the effects of an exudative process which drains the blood of a considerable quantity of salts. In typhoid fever and erysipelas a reduction usually exists which is neither so marked nor so constant. In nearly all forms of anemia the proportion of chlorides is high, following the rule above stated. Yet Limbeck found normal proportions in two cases of chlorosis, which he refers to diminished salts in the red cells, and Biernacki refers to cases of severe anemia with normal chlorides, which he also explains from the loss of red cells. Becquerel and Rodier found considerable variations, but a normal average in chlorides in six cases of chlorosis.

From the known influence upon the urinary chlorides of diminished ingestion of food, vomiting, diarrhea, and exudation, it appears probable that these factors may to some extent affect the chlorides of the blood, for in many of the available analyses of the blood ash in general diseases there are numerous contradictory results (tuberculosis, syphilis, cancer).

The phosphates exist as neutral or alkaline salts of Na, Ca, Mg, in the plasma and in various combinations (lecithin, nuclein) in the red and white cells. Few facts of importance have been established in regard to the variations in these principles of the blood.

Biernacki demonstrated a considerable diminution of P_2O_5 in anemia, and at the same time a certain parallelism between P_2O_5 and K in these conditions. In leukemia, Freund and Obermayer found an increase in P_2O_5 and Na, while the K was diminished.

The alkalies Na and K are principally combined with Cl in the blood, but Na is united in considerable proportions as phosphates and carbonates. The sodium salts, being found principally in the plasma, are subject to marked quantitative variations, being usually increased in watery blood. Potassium being located chiefly in the red cells is diminished in most hydremic conditions, and not alone in scurvy as suggested by Garrod. Sodium carbonate is probably the next most abundant salt of the plasma after sodium chloride, and to this salt mainly the plasma owes its alkalinity and its power to absorb CO_2.

Iron. The iron of the blood is principally found in the hemoglobin, a compound of albumin and iron containing, in human blood, about 0.42 per cent. of iron. It also occurs in traces in the plasma, and is found in relatively large proportion in nuclein (Hammarsten). The percentage of iron in normal blood is placed by Limbeck, from the results of several analyses, between 0.056 and 0.058. Jolles found variations between 0.0413 and 0.0559 per cent., and Hladik an average of 0.0425 per cent., using the ferrometer devised by the former.

While the principal depot of iron is in the Hb of the red cells, and the quantity of iron is closely proportionate to the percentage of Hb, the ratio between the two is often disturbed, because all iron of the blood does not form colored compounds. A further discrepancy between the Hb per cent. (after Fleischl) and the iron content of the blood results from the presence of iron-free pigments, as the *lutein* of Thudicum or the *hydrobilirubin* of Maly.

Thus Biernacki found that direct quantitative estimation always yields more iron than the computation from the percentage of Hb, after Fleischl. Sometimes twice as much iron was found as was to be expected from the Hb percentage. Similar results have been obtained by Jolles and Jellinek. It seems probable that the general introduction of a practical clinical method of estimating the iron of the blood may develop some new facts of interest in the pathology of the blood. Thus, Jellinek found in a case of *purpura hemorrhagica* that iron-free pigment was apparently absorbed from extravasations, since the Hb registered 50 per cent. after Fleischl, but only 38 per cent. as computed from the iron-content. Also in a case of malaria immediately after a paroxysm the Hb sank 10 per cent. while the iron remained constant.

Estimation of the Inorganic Principles of the Blood. It falls outside the scope of the present volume to consider the details of inorganic quantitative analysis of the blood.

For the estimation of Fe, however, reference should be made to the clinical method and results of Jolles. This method permits the estimation of Fe within fifteen minutes, and with considerable accuracy. The apparatus is offered by Reichert's agents in this country and is accompanied by full directions for use.

Mackie has described clinical methods of estimating Fe and phosphoric acid in a drop of blood, to which the reader is referred. ("Iron," *Lancet*, 1898, vol. i. p. 219; "Phosphoric Acid," *Lancet*, 1899, vol. ii. p. 484.)

Urea in the Blood.

Urea occurs in traces in normal blood (Picard) and in increased quantity in fevers (Gescheidlen), and when, as in nephritis, its excretion by the kidneys is imperfect. In uremia Munzer found 0.4 per cent. of urea in the blood, but the amount is regarded as insufficient to cause the toxic symptoms of this condition.

Demonstration of Urea in Blood. v. Jaksch recommends the following procedure: 200 to 300 c.c. of blood are precipitated with three to four times as much alcohol, and after twenty-four hours the precipitate is repeatedly washed in alcohol. The alcohol is then evaporated, the residue treated with nitric acid, and the crystalline mass, secured after some hours, is dried between filter paper, dissolved in water, treated with barium carbonate till CO_2 ceases to form, dried on a water-bath, and extracted with hot alcohol. On evaporation urea is found in slender rhombic prisms. If secured in sufficient quantity the crystals may be treated with nitric acid and evaporated, when characteristic crystals of urea nitrate form. Or the biuret test may be employed, dissolving the crystals in a little caustic potash, and adding a drop of dilute cupric sulphate.

Various other methods may be found described in text-books of physiological chemistry—*e. g.*, Gamgee.

Uric Acid in the Blood.

Scanty traces of uric acid have been demonstrated in normal blood (Picard, Abeles). Garrod found 0.025 to 0.145 per cent. during acute attacks of gout, and Salomon also found an increase in acute gout. v. Jaksch regards their methods as inexact. A moderate increase (0.08 per cent.) has been demonstrated in pneumonia and anemia (Salomon,[1] v. Jaksch[3]), in cardiac and other forms of dyspnea, and in nephritis (v. Jaksch). Klemperer[1] and Weintraud noted moderate increase in leukemia (0.09 per cent.), in nephritis (0.06 per cent.), and in uremia (0.19 per cent.), but failed to find any trace in three healthy subjects and in one case of pneumonia.

Estimation of Uric Acid.—Garrod took 10 c.c. of serum from 30 c.c. to 35 c.c. of coagulated blood, added 10 per cent. of dilute acetic acid, and obtained crystals of uric acid on a thread placed in the fluid. In blood containing not less than 0.025 per 1000 of uric acid the thread was covered with crystals in twenty-four to forty-eight hours. These crystals should be submitted to the murexide test.

v. Jaksch recommends the qualitative and quantitative estimation of uric acid by means of the Ludwig-Salkowski method employed in urinary analysis. The blood is prepared for this method as follows: 100 c.c. to 300 c.c. of blood are diluted three to four times with water, heated on the water-bath until coagulation begins, when it is feebly acidified by acetic acid (s. g. 1.0335). After fifteen to twenty minutes it is filtered, the precipitate washed with hot water, boiled again after feebly acidifying, cooled and filtered. A little sodium phosphate is now added to the filtrate, which is then submitted to the Ludwig-Salkowski method. For a full description of this method, see Simon's *Clinical Diagnosis.*

Glucose in the Blood.

Normal blood always contains traces of glucose, which may be increased by diet rich in carbohydrates, and is diminished by muscular exercise and hunger (Seegen, Chauveau and Cavazanni, v. Mering). Limbeck found in the blood of two healthy subjects, five hours after eating, 0.075 per cent. and 0.089 per cent. of glucose. Freund and Trinkler find that glucose is very considerably increased in the blood in cases of carcinoma, readily reducing cupric oxide when freed from albumins, which cannot be accomplished with the blood of any other disease except diabetes. In one case of cancerous cachexia Trinkler found 0.3 per cent. of glucose, which reaches the limit stated by Claude Bernard to be capable of producing diuresis.

In diabetes the quantity of glucose in the blood is subject to great variations, according to the character and progress of the disease. Hoppe-Seyler found in one case 0.9 per cent.

Estimation of Glucose in the Blood. A weighed quantity of blood is freed from albumin by boiling with an equal quantity of sodium sulphate and filtering. The precipitate is well washed and the presence and quantity of glucose in the filtrate determined by Fehling's solution or by polarimetry. A certain amount of sugar is carried away by the precipitated albumins, and unless the blood is fresh, the action of the glycolytic ferment may cause serious error in the result.

Glycogen in the Blood.

The long discussion regarding the feasibility of demonstrating glycogen in the blood by chemical methods has led to many contrary opinions, but in recent years positive results have been more constant (Salomon, Frerichs, Cramer, Lepine and Barral, Huppert, Czerny).

The *microscopic test* on specimens dried in the air and stained by iodine, after Ehrlich's suggestion, has given more uniform results. Gabritschewsky, using this method, found intracellular and extracellular glycogen in the blood of both healthy and diseased persons. *Extracellular glycogen*, in the form of fine or coarse granules (1μ to 6μ) he found to be the only form usually present in normal blood; and it was increased in diseases in which intracellular glycogen was abundantly present. He states that extracellular glycogen is derived from the disintegration of leucocytes, but offers no evidence to prove this origin. The glycogen was found in the bodies of neutrophile leucocytes in cases of diabetes and leukemia, and in the plasma in a considerable variety of other diseases. The results of his experiments, injecting sugar and peptone into the blood of animals, indicate that the leucocytes are capable of transforming both sugar and peptone into glycogen. Livierato also found extracellular glycogen in normal blood, but failed to discover any in the leucocytes in diabetes. From his clinical observations he concludes that the glycogen of the blood is increased in febrile cases with an active exudative lesion and with leucocytosis. In typhoid fever he found only extracellular glycogen.

Czerny's studies added considerably to the knowledge of the subject. He found the increase of glycogen in exudative processes with leucocytosis. In the blood of children with cachectic leucocytosis he found an almost equal quantity. In two or three dogs exposed to prolonged cold he found that glycogen appeared in the leucocytes after twenty-four hours, and persisted for several days. From the severe disturbances of respiration following section of both vagi, or pneumothorax artificially introduced, as well as in severe anemia from hemorrhage, he found a marked increase in glycogen. An interesting inquiry of Czerny's related to the exact chemical nature of the brown staining granules demonstrated by iodine, a reaction common to both glycogen and amyloid. That these brown granules are glycogen is indicated by (1) their reaction to iodine; (2) by the disappearance of their brownish stain on heating (Barfurth), and (3) by their complete digestion by saliva (Czerny). On the other hand, Czerny points out that pure glycogen is soluble in water, while the glycogen of leucocytes is insoluble in water (as the writer, also, has found), that iodine with dilute sulphuric acid colors these granules violet, which is the reaction of amyloid; and, finally, that in dogs suffering from prolonged suppuration whose blood continually shows abundance of "glycogen," the viscera, on the other hand, develop extensive amyloid changes. These observations indicate that the brownish staining granules are not pure glycogen, but a comparatively insoluble compound of glycogen with some other substances, or else an intermediate product between glycogen and amyloid.

Caminer failed to find any glycogen in normal blood, and in disease very rarely found it in the plasma. He distinguished three stages of the deposit in leucocytes: (1) the presence of a light, diffuse mahogany stain; (2) the presence of a few isolated globules; (3) the complete transformation of the body of the cell into glycogen. In cases of extreme sepsis, all stages were seen; in pneumonia glycogen was usually present, but never in the third stage of its formation; in phthisis only the first stage was found; and in rheumatism it was absent. In cases of carcinoma it was found only when suppuration occurred. In four diabetics none was found, but in a case of diabetic coma it was abundant. It was absent in most cases of carcinoma, in chlorosis, in two cases of leukemia, and in secondary anemia from hemorrhage. From experimental studies, he finds that three factors are concerned in the glycogenic degeneration of leucocytes: (1) fever, (2) leucocytosis, (3) toxemia, of which the last is most potent.

T. Dunham found the glycogenic degeneration of leucocytes a valuable aid in the diagnosis of suppuration, and introduced the term "iodophilia." Locke has contributed extensive clinical observations on the occurrence of glycogen in leucocytes. He found an extracellular reaction, especially in the blood plates, in all cases, and regards this occurrence of no clinical importance. In septicemia an intracellular reaction was marked and constant. In abscess it was present in 22 of 25 cases, but disappeared in forty-eight hours after drainage of the pus. The three negative cases were very small abscesses without fever or leucocytosis. In appendicitis, peritonitis, and empyema it was abundant when pus was forming, but otherwise absent or slight. In serous pleurisy no reaction occurred even with high fever. In 49 cases of pneumonia it was constant and persistent. In malaria 22 of 29 cases gave a positive reaction. In 17 of 42 cases of typhoid fever the reaction was present, but referable to inflammatory complications. After perforation the reaction increased within six hours. In tuberculosis it was absent or faint if there were no suppurative complications. In diabetes a positive reaction occurred only with coma or gangrene. In pernicious anemia a very faint reaction was occasionally seen.

Numerous other reports on the occurrence of glycogen in leucocytes have appeared, showing that the subject is of considerable clinical interest and sometimes of value in diagnosis. Regarding the microscopic demonstration of extracellular glycogen, it should be mentioned that other principles, such as myelin, lecithin, and amyloid, stain brown with iodine.

Lipemia.

The occurrence of free fat (palmitin, stearin, olein) in the blood, both in health and in disease, has repeatedly been observed. While usually present in such small quantity as to be recovered in very small amounts from the ethereal extract, and recognized with difficulty by the microscope, it is sometimes so abundant as to give the blood a milky appearance (Gumprecht). *Its physiological variations probably exceed the pathological, as it has been found very much increased in healthy individuals after a hearty meal.* In disease its occurrence appears to follow no general rule, so that its real pathological significance remains doubtful. v. Jaksch, extracting the blood with ether and thereby including fat, lecithin, cholesterin, and a trace of nitrogenous compounds, found in three cases of diabetes 0.05 to 0.16 per cent., in nephritis 0.1 to 0.5 per cent., in typhoid fever 0.16 per cent., and in pneumonia 0.15 per cent. It has also been found in increased quantity in phthisis, poisoning by carbonic acid, and in fat embolism after traumatism.

Englehart found considerable variations in the ether extract of human blood in healthy subjects, viz., 0.101 to 0.273 per cent., average 0.194 per cent., while in cachectic subjects, although no greater variations were found, the average was lower than 0.174 per cent. In fasting dogs, Cohnstein and Michaelis found an average of 0.121 per cent. in seven analyses, while after fatty diet the average rose to 0.153 per cent.

Acetonemia. Lipacidemia.

Deichmuller and v. Jaksch, by extracting the blood with ether and by distillation, have isolated a principle which gives the reaction of acetone, and which they found increased in many processes, especially in fevers. Fatty acids have been found in the blood by v. Jaksch, in diabetic coma, leukemia, acute yellow atrophy of liver, and acute infectious diseases: β-oxybutyric acid, in the cadaveric blood of diabetes, by Hougounenq; and sarcolactic acid, in normal blood, by Gaglio, Spiro, and Berlinerblau. Limbeck doubts the reliability of these results, believing that fatty acids may develop from lecithin during the technical procedures followed in isolating these principles.

Cholemia.

The poisonous symptoms developed in cholemia have been referred by most authorities to the presence of biliary acids. Flint's statement that cholesterin is the poisonous agent has not been accepted, although it has been found in considerable traces in icteric blood.

To the naked eye icteric blood may appear of yellowish red color, while in the serum or its foam small quantities of bile pigment are readily detected by the peculiar yellowish tinge. On repeated heating the yellowish red bilirubin changes to the green biliverdin.

Diminished isotonic tension and increased resistance of the red cells are a peculiar character of the blood in jaundice. Limbeck found the tension of the cells reduced 0.4, 0.38, and 0.32 per cent. NaCl, and that of the serum 0.76 and 0.864 per cent. NaCl. The well-attested fact that in intense jaundice red cells are frequently dissolved by biliary principles, can, with some difficulty, be reconciled with this markedly hyperisotonic quality of the serum. Limbeck believes that bile acids affect the union of Hb with the stroma of the red cells, rendering the Hb more easily soluble, and thinks that the solution of red cells in jaundice, as well as in other conditions, depends on other than simple osmotic factors.

Other characters of icteric blood are, according to Limbeck's analyses, an increase of nitrogenous bodies (3.29 to 3.52 per cent.); diminution of chlorides of both blood and serum, which he refers to their displacement by biliary acids; and a well-marked increase in the volume of red cells (Bleibtreu's method).

Detection of Biliary Principles in the Blood. Well-marked cholemia may be detected by naked-eye inspection of serum or its foam. On heating to 50° C. bilirubin may be changed to biliverdin.

v. Jaksch has been able to demonstrate bilirubin in the blood when none was to be found in the urine, or when only urobilin existed in the urine, by the following procedure: A little blood obtained by a wet-cup is allowed to coagulate, and after one to two hours the serum is drawn off and forced by aspiration through an asbestos filter. The froth of the filtrate is yellow if any bilirubin is present, and this yellow tinge becomes green on heating two or three hours at 35° C., if any minute traces of bile pigment are present. Or the blood may be coagulated slowly at 78° to 80° C. when the serum becomes greenish in the presence of minute traces of bile.

Biliary acids may be demonstrated by Pettenkofer's method on serum prepared as follows:

Albumins are removed by boiling or by alcohol, and the filtrate is treated with lead acetate, and with ammonia, which precipitates the acids with the lead compounds. The acids are then recovered, by washing the precipitate on a filter, boiling in alcohol, filtering, and decomposing the lead salts by carbonate of soda. The solution is again filtered, evaporated to dryness, and the acids extracted by boiling in absolute alcohol. Finally, on evaporating the alcoholic extract, biliary acids crystallize out, or an amorphous substance remains, from which the crystals may be obtained by extracting with ether.

The Specific Gravity of the Blood.

The specific gravity of the blood changes with the content of water, its most variable constituent, the proportion of salts, which are less variable, and the percentage of albumins, which are the last principle affected by pathological processes.

The *normal limits* have been placed by Becquerel and Rodier between 1.058 and 1.062 for men, 1.054 and 1.060, including both sexes; by Hammerschlag, between 1.056 and 1.063, including both sexes; by Lloyd Jones, between 1.045 and 1.066. Some of these discrepancies are doubtless referable to the differences between the methods employed.

There are considerable *physiological variations* in gravity. According to Lloyd Jones the blood of newborn infants shows the highest gravity, averaging 1.066; after the second week of life up to the second year the gravity sinks, 1.048 to 1.050; rising with men between thirty-five to forty-five years to 1.058, with women after the climacteric, to 1.054; in old age the blood of both sexes approaches the initial high gravity of infancy. Limbeck finds an explanation of these progressive changes in the decreasing capacity of the tissues to absorb water. From forty-four estimations on his own blood Schmaltz found minimal variations *at different hours of the day*, from 1.061 from 7 to 8 A.M., to 1.058, from 2 to 8 P.M. Muntz found a marked increase, 1.038 to 1.058, in the gravity of the blood in overfed sheep. Muscular activity, if accompanied by sweating, slightly decreases, sleep slightly increases the gravity (Schmaltz, Jones). Schmaltz found that menstruation is followed by a slight increase of gravity, while the slightly lower gravity of pregnant and parturient women has been frequently observed.

For somewhat obscure reasons the gravity of the blood is increased by residence in *high altitudes* and a considerable difference (0.015) has been observed in the blood of animals pastured on mountain tops and those grazing in the valleys (Muntz, Viault, Glogner).

In pathological conditions lowered specific gravity is a constant character of the blood in anemia.

In *chlorosis* the change is usually referable to and proportionate with the loss of Hb, but Stintzing and Gumprecht and Siegel have shown that this parallelism is not invariable. In *pernicious anemia* the specific gravity and dry residue suffer more than the Hb, owing to loss of albumins from the serum. Extremely low gravity, in comparison with the Hb-content, is characteristic of this condition. In *leukemia* the gravity is reduced as in simple anemia, but extreme reductions are seldom observed, owing to the increase of white cells and the presence of abnormal products soluble in the plasma. In *secondary anemias* there are numerous exceptions to the parallelism between specific gravity and Hb-content. These are found especially in diseases in which an exudative process drains the blood of albumins (dysentery), or when from edema there is relative hydremia of serum or red cells.

In the *infectious diseases* the specific gravity of the blood depends upon associated conditions, such as profuse perspiration, diarrhea, exudation, etc., more than upon any specific property of bacteria to increase the water of the blood. That the power to impoverish the blood varies, however, with different infectious agents is strongly indicated by clinical observation. Grawitz's claim that tuberculin and the diphtheria toxin tend to increase the gravity of the blood, while the toxins of *streptococcus* and *staphylococcus pyogenes* tend to diminish it, is frequently confirmed by comparison of the blood in cases of miliary tuberculosis and uncomplicated diphtheria with that of septicemia.

Although acute stasis is usually followed by marked increase in the gravity of the blood, yet in *chronic endocarditis* the variations observed are very irregular. General edema is usually associated with lowered gravity.

The same observation applies to the blood of *nephritis*, normal gravity being observed in many cases of chronic interstitial nephritis (Hammerschlag), while anemia and low gravity of the blood and edema of the tissues are nearly constantly associated with the large white kidney.

Numerous studies of the blood of *pulmonary tuberculosis*, including that of Grawitz, have failed to bring to light any uniform variations in gravity peculiar to the blood in this disease. As a rule, extreme reductions are not observed.

In the cachexia of *malignant neoplasms* some of the lowest gravities on record have been observed (1.030, 1.032, Lyonnet), especially in ulcerating and bleeding tumors of the stomach and uterus.

In certain *skin diseases* (pemphigus, eczema, psoriasis, prurigo, *morbus maculosus*) an increased gravity of the blood has been noted by Schlesinger. In cases of general burns, Tappeiner, Baraduc, and Schlesinger have found very high gravities, 1.065 to 1.073, which, in cases that recovered, fell to normal in twenty-four hours.

Finally, *various drugs* appear to exert a moderate but in no sense peculiar influence on the gravity of the blood. Purges, diaphoretics, and diuretics remove water from the system, and when this loss is not immediately replaced from the tissues the blood is temporarily concentrated. The action of mercury is somewhat uncertain, but in syphilis it appears to diminish the gravity after a short initial period of increase (Schlesinger).

To recapitulate, it has been found that considerable variations in the water of the blood may under ordinary conditions be promptly equalized by the action of the tissues on the one hand replacing a loss, or of the kidneys and skin removing an excess, so that the change in the blood is very transitory. Only when there is interference with these processes or when the demands upon them are excessive does a more or less permanent alteration in the gravity of the blood follow.

On the other hand, when the albumins of the blood are affected, more marked and permanent changes are produced. In general, the content of the tissues in water and that of the blood are very closely interdependent.

The Ferments of the Blood.

In recent years the metabolic processes in the cells, both catalytic and synthetic, have been referred more and more to the agency of ferment-like bodies, many of which have been demonstrated in the blood. It falls outside the scope of this work to consider the relations of the fibrin ferment and of the probable ferment action of the toxophore group of Ehrlich's complement, but some other special fermentative processes inherent in the blood or its cells deserve brief mention.

Oxidase. The presence of an oxidizing ferment in the blood was first demonstrated by Schonbein, who located the ferment in the stroma principally of the red cells. Its importance in physiology is now fully recognized.

Lillie has applied an interesting method of demonstration of an oxidase in leucocytes and red cells. When blood is mixed with an alkaline solution of α-naphthol or paradiamidobenzene, in equimolecular proportions, the solution, at first colorless, becomes deeply tinted from oxidation, and many stained granules of oxidation products are deposited about the nuclei of leucocytes and in the red cells.

Lipase. The presence of a fat-splitting ferment in the blood has been claimed by Hanriot and others, but its existence is not fully proven (Jacoby).

Proteolytic Ferment. Ascoli and Moreschi report that the washed sediment of sterile pus from empyema contains a proteolytic ferment. To the action of such a ferment in the leucocytes has been attributed the dissolution of the pneumonic exudate, in which, of course, the leucocytes are mingled with other cells and are dying.

Glycolytic Ferment. The presence of a glycolytic property in normal blood has been long known. Lepine found that it depends on the red cells more than upon the plasma, and he elaborated the theory that diabetes results from the diminution or absence of this glycolytic ferment. It has been shown, however, that the glycolytic property of diabetic blood is not distinctly inferior to that of normal blood (Minkowski, Kraus). The experiments of Seegen and Arthus indicate that this property is not exerted during life, but is a cadaveric phenomenon, and that the extent of its action depends upon the time consumed in the examination of the specimen. This objection applies to present views regarding nearly all of the cell ferments and is not generally regarded as valid (Jacoby).

Spitzer, in an exhaustive study, finds that a glycolytic ferment is present not only in red and white blood cells, but in all tissue cells; that it is precipitated in active form by alcohol, and that its action is possible only in the presence of oxygen as contained in oxy-Hb.

Diastatic Ferment. The study of the property of fresh blood to digest starch has lately been extended to the blood in various diseases. This property may be demonstrated by adding 1 c.c. of blood to 50 c.c. of a solution of starch, allowing the mixture to stand a few hours in the thermostat, when glucose may be demonstrated by Fehling's solution.

Castellino and Pracca found that 2 c.c. of normal human blood added to 50 c.c. of starch solution produced 0.07 per cent. of sugar in twenty-four hours, at 30° C. Arterial blood is more active than venous, and the fermentation most active between 30° and 38° C., is inhibited at 75° C., and diminished by slight changes in reaction (Cavazanni). Cavazanni and Pracca found considerably increased fermentative power of the blood in anemia, chlorosis, leukemia, pneumonia, malaria, nephritis, cirrhosis, and carcinoma, while in other cases of these and other diseases it was diminished.

The principal source of the diastatic ferment is placed in the red cells by Tiegel and Plosz, in the serum of Bial, and in the leucocytes by Castellino and Pracca. The last-named observers find that the diastatic property is closely related to the globulicidal and coagulative power of the blood; that it increases after the blood is shed; that it is inhibited by the addition of nuclein, and increased by sodium sulphate and chloride.

Tschereskoff was able to precipitate from blood by alcohol small amounts of a diastatic ferment which remained active forty-five days. It was more abundant in the serum than in the clot, and the presence of sodium oxalate did not destroy it.

The Osmotic Relations of the Blood.

When a drop of blood is placed in distilled water the red cells are promptly dissolved, but when blood is placed in a solution of salt of a certain concentration the red cells retain their Hb, and sink to the bottom of the fluid. The solution of the cells results from the law of osmosis, that when two solutions of different concentration are separated by an animal membrane the solutions pass through the membrane until the quantity of salt in each is equal. The force which leads to this interchange is called "osmotic tension," and two fluids with equal content of salt are said to be "isotonic," or of equal "isotonic tension." Fluids are likewise said to be *hyperisotonic* or *hypisotonic* when they contain greater or lesser quantities of diffusible salts than other fluids, and are capable of drawing water from or yielding it to such fluids, according to the laws of osmosis.

A solution containing 0.46 per cent. of NaCl is just sufficient to prevent the solution of red cells in the average specimen of normal human blood (Limbeck), *and the isotonic tension of human red cells may, therefore, be said to be 0.46 per cent. NaCl.* Yet when red cells are placed in a 0.46 per cent. solution of salt they absorb water and swell, although they do not yield up Hb, and when placed in strong solutions of salt, red cells shrink, yielding water to the fluid. *Hamburger finds that a 0.9 per cent. solution of salt causes neither swelling nor shrinkage of the red cells. This solution, therefore, represents the isotonic tension of the blood plasma, and is properly called the "normal salt solution."*

Any considerable lowering of the osmotic tension of the plasma must therefore lead to swelling of the red cells and eventually to their solution. The hyperisotonic quality of the plasma with reference to the red cells is a physiological necessity, otherwise the ingestion of a considerable quantity of water would cause the solution of many red cells. The exact limits of osmotic tension between which the red cells suffer no alterations of volume are not known, but it is certain that they are often exceeded in pathological conditions. Changes in osmotic tension which affect the volume of the red cells may occur not only in the plasma but also in the red cells. Thus, if the red cells in chlorosis are for some developmental anomaly deficient in salts they would shrink in plasma of normal (0.9 per cent.) osmotic tension, while normal cells would swell in the watery plasma supplied after hypodermoclysis for hemorrhage.

The isotonic relations of the blood do not apparently depend entirely upon the presence of salts, but are affected also by the presence of other diffusible principles, as the albumins. Limbeck finds only 0.2 per cent. of salts in red cells, yet their isotonic tension is equivalent to at least 0.46 per cent. NaCl.

Hamburger finds that albumins, phosphates, and chlorides behave differently under changing osmotic conditions. When a little acid is added to blood, albumins and phosphates pass from red cells to serum, while chlorides pass from serum to cells, but when alkali is added the opposite transfer is induced. Similar physical effects are produced by the passage of oxygen and carbonic acid through the blood, and Hamburger suggests that these factors take important part in the metabolic exchanges in the capillaries.

One of the chief physiological relations of the isotonic tension of the blood is its influence in confining Hb to the red cells.

Hamburger, who was one of the first to study this subject, regarded the fixation of Hb as the result solely of osmosis on the fluid Hb lying within the membrane of the red cell. Yet the opinion of Hoppe-Seyler that Hb enters into chemical or molecular union with the stroma of the red cell, and the fact that the existence of a membrane about the erythrocyte has not been satisfactorily proven (Limbeck) render this belief uncertain. Limbeck offers evidence to show that there are other than purely physical influences concerned with this important function, viz., the chemical union of Hb with other elements of the red cells, and the influence of albumins on osmosis.

Physiological variations in isotonic tension of the blood are numerous. That of venous is slightly higher than that of arterial blood (0.02 per cent.). The addition of CO, CO_2, hydrogen, nitrogen, arsenic, or a trace of acid increases isotonic tension, while oxygen and traces of alkali diminish it (Limbeck). In pathological conditions, from a series of fifty-four observations on blood from venesections, Limbeck concludes: During acute infections, especially typhoid fever, erysipelas, and pneumonia, the isotonic tension of the blood is frequently much increased, but not always or constantly so. In general disturbances of nutrition the tension of the blood is very variable; in diabetes and osteomalacia it was normal; in leukemia it was much increased; in jaundice it was low, while in chlorosis it was low, and in severe secondary anemia higher than normal.

In pregnancy and lactation Vicarelli found a distinct increase in osmotic tension—*i. e.*, a diminished resistance of the red cells to water (0.6 to 0.66 per cent. NaCl). While Limbeck and Castellino found the red cells less resistant in typhoid fever, Bianchi and Mariotti found that experimental injections of *B. typhosus* lowered the isotonic tension of the blood, although filtered cultures of this and other bacteria had the opposite effect. Cavazanni found that injections and inunctions of mercury slightly increased the resistance of the red cells.

The *tension of the serum* has been investigated by Hamburger, using another method, who found no change after bleeding. Viola and Jona, during seven hours following venesection, found a moderate diminution, while Limbeck, in three portions of blood, taken at intervals during the exsanguination of a dog, found nearly constant conditions, and Adler found little variation in the tension of the serum in various diseases.

It will thus be seen that the knowledge of this subject is yet in a very rudimentary condition; although its importance in hematology invites further investigation.

Alkalescence of the Blood.

It is an established principle of physiology that the capacity of the blood to absorb CO_2 depends on its alkalescence.

When one compares the results of direct alkalimetry obtained by any of the recognized methods with the proportion of CO_2 obtained after the dissociation of carbonates by strong acids, marked discrepancies are observed. The volume of CO_2 differs greatly from the degree of alkalinity obtained by direct titration. To explain this fact it must be supposed that certain basic properties of the blood are brought into action by alkalimetric methods which are not active during life, or else that under changing conditions of metabolism the blood is required to absorb varying quantities of CO_2, which are by no means necessarily proportional to the capacity of the blood to absorb this element.

There are good physiological grounds for supposing that both of these conditions actually exist. It is probable that the existing methods of titration are sensible to the alkaline carbonates and phosphates, which are principally concerned in the alkalinity of the blood, and, as well, to some acid-neutralizing albuminous principles that are liberated from plasma and red cells during alkalimetric procedures (Limbeck and Steindler). To what extent the phosphates and albumins are concerned in the physiological functions centred in the alkalinity of the blood is not known, but it has seemed to the writer that the opponents of the titration method have failed to consider the possibility that other important functions besides that of absorbing CO_2 may depend on the alkalinity of the blood (*e. g.*, fibrin formation), and that the results of the titration method, therefore, deserve recognition in the study of the pathology of the blood.

On the other hand, the attempt to measure the alkalescence of the blood by the volume of CO_2 recovered after addition of acids involves a needless confusion of the problem, as such estimations include both the CO_2 loosely combined with Hb and that more firmly united with the alkalies. Yet the physiological significance of each of these combinations is probably very different, the former representing the respiratory activity of the blood and the metabolic activity of the tissues, while the latter is related to other less definitely known functions. Moreover, it by no means follows, as has been said, that the volume of CO_2 recovered from the blood represents the total quantity that the blood is capable of absorbing, or is any indication of its acid neutralizing power. Bunge calculates that after allowing for the amount of sodium required to saturate the only strong mineral acid of the plasma (HCl), there is enough sodium left to fix 63 volumes per cent. of CO_2 as carbonate and an equal additional amount as bicarbonate, which is far more than the amount of CO_2 actually present in the blood.

Again, the attempt to estimate the alkalescence of the blood, considered from either the biological or chemical standpoint, by the content in CO_2 involves several probable errors. There may, first, be a diminished production of CO_2 in the tissues in pathological conditions. There may be a diminished absorption of CO_2 by the blood, owing to chemical changes in the tissues or mechanical impediments in the circulation. Finally, there may be simple absorption of CO_2 uncombined with acid neutralizing principles (Schafer).

Of the actual existence or importance of these factors, it is, with the present knowledge of physiology, difficult to judge, but since they stand as uncertain quantities, it seems unwise to rely upon any such indirect method of judging of the alkalinity of the blood.

It would seem, therefore, that both alkalimetry and the estimation of CO_2 furnish important information in regard to the state of the blood, but there are no good *a priori* reasons to suppose that both measure the same property of the blood, while the practical results obtained positively disprove such a view. When laked blood is titrated a high degree of alkalescence is obtained, as this method takes account of all acid-neutralizing principles, carbonates, phosphates, and albumins of plasma, and red cells. When serum is titrated, the acid neutralizing principles of red cells are ignored, and lower grades of alkalinity are obtained. When the CO_2 is estimated account is taken only of the carbonates, but albumins and phosphates are ignored, and the presence of any dissolved CO_2 disturbs the computation, while the possibility still remains of accidental variations in the ratio between the CO_2 actually present and total capacity of the blood to absorb this acid.

Brandenburg distinguishes between the total alkalescence of the blood and its "alkaline tension," the latter signifying the diffusible alkalies, the former those in non-diffusible combination with albumins. The percentage of diffusible alkali increases as the albumins of the blood diminish. In normal blood he found about 20 per cent. of the alkali diffusible, in chlorosis about 33 per cent. The freezing point of blood was considerably influenced by the diffusible alkalies, but very little by those combined with albumin. (See Diabetes.)

The studies of the CO_2-content of the blood have given the following chief results, as summarized by Limbeck:

Venous blood is always richer than arterial in CO_2, and both are subject to minor physiological variations, 33.37 to 45.3 volumes per cent. (Schafer, Kraus).

Febrile processes are generally accompanied by diminution of CO_2 in the blood, often in proportion to the height of the fever, 34.18 to 20.9 volumes per cent. (Geppert). This condition, according to Geppert and Minkowski, is referable to the abnormal production of acid metabolic principles of the blood, a view which is supported by Kraus, who, in fevers, along with a diminution of CO_2 (10 to 20 volumes per cent.), found an increase in the acid principles of the blood.

In *the cachexia of carcinoma* Limbeck and F. Klemperer found marked diminution of the CO_2 of the blood (9.67 to 20.5 volumes per cent.), but did not estimate the acid principles. Similar results furnished by other observers indicate that in diabetic coma the blood is frequently very deficient in CO_2 (Minkowski, Stadelman, Kulz, Hallervorden, Kraus). Yet Kraus in one case found a normal quantity. A simultaneous increase in acid principles has been demonstrated in one case of diabetic coma by Kraus, and on these grounds it has been concluded by various authors that in diabetes there exists an acid intoxication probably from oxybutyric and diacetic acids. Limbeck accepts this view and concludes from the various studies that in acute febrile infectious diseases, in cancerous cachexia, and in diabetic coma, a diminution in CO_2 and an increase of acid principles of the blood have been fully demonstrated, pointing in all probability to the existence of an acid-intoxication in these diseases. In *leukemia* Kraus[2] found a slight deficiency of CO_2 (20.29 volumes per cent.).

From the direct alkalimetry of the blood or serum much less uniform results have been obtained. A fairly constant normal alkalinity has been established with *titration methods*. v. Jaksch[5] placed the alkalinity of normal blood at 0.26 to 0.30 per cent. NaOH; Kraus, at 0.226 per cent.; Jeffries, at 0.2 per cent.; Drouin, at 0.206 per cent.; Freudberg, at 0.2 to 0.24 per cent.; Limbeck, 0.220 to 0.256 per cent. In laked blood it has been found much higher by Loewy, 0.449 per cent.; by Berend, 0.45 to 0.5 per cent. Peiper found slightly greater alkalinity in the blood of women than in that of children, and in the blood of men over that of women. Berend and Preisich, using Tausczk's method, found the alkalinity of the blood to be very high at birth and for the first six months, after which it diminishes rapidly, reaching its lowest point in the second year. After the third year it increases until at the sixteenth year it reaches the normal degree of adult life. A constantly diminished alkalinity in the blood during fevers has been reported by the above authors, by Rumpf, and others, and in carcinoma (Rumpf), anemia (v. Jaksch, Peiper), leukemia (Peiper, Rumpf), uremia, cirrhosis, and osteomalacia (v. Jaksch). On the other hand, the results obtained by Loewy and by Limbeck and Steindler, using their particular methods, were extremely variable, and these variations were about equal in health and disease.

From the observations of Fodor, Behring, Zagari, Calabrese, Cantani and Rigler it has been shown that during infectious diseases the alkalinity diminishes as the disease progresses unfavorably, reaches its lowest point just before death, and that the alkalinity of the blood is essentially connected with the natural or acquired immunity of the animal organism against bacterial infection. Fodor found that immunization of animals against anthrax, cholera, and swine plague increased the alkalinity of the blood. No increase followed injections of bacillus typhosus or tuberculosis. Burmin found that the alkalescence of the blood in anemia varies directly with the red cells.

Using his own method Rigler has recently contributed an elaborate study, with the following main conclusions:

The alkalinity of normal rabbit blood does not vary more than 6 per cent. After infection by various pathogenic bacteria, including typhosus and tuberculosis, especially if fatal, there is uniform and usually marked diminution in the alkalinity of the blood. As recovery from the infection follows the alkalinity increases. Similar results followed injections of various bacterial toxins, of phosphorus, potassium chlorate, picric acid, biliary acids, pilocarpin, and atropin.

He found, as did Cantani and Fodor, that injections of diphtheria antitoxin, anthrax vaccine, etc., causes a marked but temporary increase of alkalinity. In rabbits treated by injections of serum of various animals considerable variations in the alkalinity of the blood were observed.

In twenty-three patients suffering mostly from infectious diseases Rigler found similar variations with those observed in animals.

The Acidity or Basic Capacity of the Blood.

There are certain unsaturated salts in the blood, $NaHCO_3$, NaH_2PO_4, and probably Na_2HPO_4 (Limbeck), which although alkaline to litmus are acid to phenolphthalein, and are capable of uniting with bases. While fresh blood is alkaline, serum reacts as acid to phenolphthalein. The capacity of the blood salts to neutralize bases has been called its *basic capacity* by Kraus,[2] who devised a delicate and somewhat difficult method of measuring this capacity.

In normal venous blood Kraus found a basic capacity of 0.162 to 0.232 per cent. NaOH, which increased in febrile conditions to 0.209 to 0.272 per cent. NaOH. His demonstration of a marked increase of basic capacity (0.347 per cent.) in diabetes has been regarded as strong evidence of an acid intoxication in this disease.

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CHAPTER III.

MORPHOLOGY AND PHYSIOLOGY OF THE RED CELLS.

In fresh normal blood the red cells appear as homogeneous biconcave disk-shaped bodies, with opaque yellowish rims and nearly transparent centres. In the capillary circulation they exhibit remarkable elasticity, folding, indenting, and greatly elongating, without rupture. After early infancy they are invariably non-nucleated. They show a strong tendency to cohere to one another by their flat surfaces, forming long rows (rouleaux). This property has been referred to the presence of a fatty material surrounding the red cells.

Brunton placed cork disks covered with soap in acidulated water, and found that they formed rouleaux, probably with the liberation of fatty acids. Rouleaux formation by red cells, he suggests, is probably due to the liberation of fatty acids under the influence of CO_2. Peskind has shown that agglutination of red cells is produced by various acids and acid salts through alteration in the alkalinity, and combination with the nucleoproteid, of the cell envelope.

In dry specimens when thinly spread and rapidly dried the cells are circular in outline, their concavities are obliterated, and they stain evenly throughout. When rather thickly spread the rouleaux are retained, the concavities persist, and the centres may be nearly transparent while the thicker rims are densely stained.

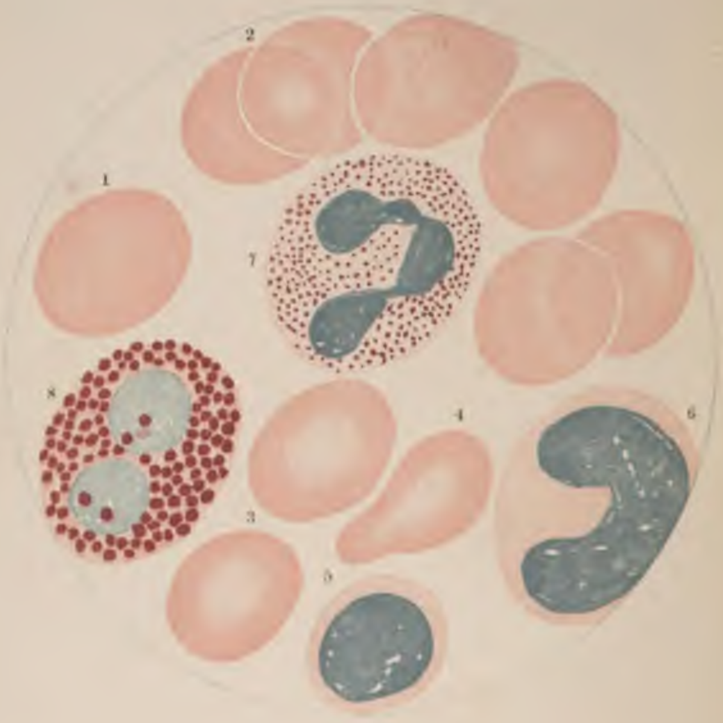
Neither *membrane* nor *reticulum* have been fully demonstrated in the human red blood cells, although both, from analogy, have been supposed to exist. It is more probable that the hemoglobin is held in compact form principally by a chemical union with other albuminous constituents of the cell (Schafer).

Peskind's studies indicate that the red cells are enveloped by a hemoglobin-free layer composed of lecithin, cholesterin, and a nucleoproteid.

Staining Reactions. The living red cell is achromatic, but when fixed is markedly acidophile in quality, a property probably residing exclusively in the Hb. In the centre of the cell there is an achromatic constituent which fails to stain with acid dyes, but under some circumstances this central substance may become partially separated or even extruded from the cell, when it stains lightly with methylene blue, and yields the specific reaction of chromatin (Nocht's method).

Two views regarding the structure of the red blood cell have been maintained. According to the first, the red blood cell is vesicular, consisting of colored semifluid contents, hemoglobin, surrounded by a membrane and supported by a stroma composed of various substances, principally lecithin, cholesterin, and cell-globulin (Schafer). According to the second view, the red cell is not vesicular, but a viscous solid mass, consisting of a colorless

PLATE I.



Normal Blood. (Triacid Stain.)

Fig. 1. Normal red cell, flatly spread, and evenly stained.

Fig. 2. Normal rouleau.

Fig. 3. Normal red cells, varying slightly in size, thickly spread, showing central clear area.

Fig. 4. Normal red cell, of slightly altered shape.

Fig. 5. Lymphocyte, medium size.

Fig. 6. Large mononuclear leucocyte, incurved nucleus.

Fig. 7. Polynuclear neutrophile leucocyte.

Fig. 8. Eosinophile leucocyte. Separate nuclear lobes.

elastic stroma which is condensed at the periphery and which supports, partly by mechanical means and partly by chemical union, the hemoglobin and other constituents of the cell (Rollet, Brucke).

It is not impossible to partly reconcile these opposing views regarding the peripheral condensation of stroma as in many respects similar to a membrane. That the cell is not strictly vesicular with separable fluid contents is shown by the fact that it may be subdivided without escape of contents, each fragment assuming the discoidal shape.

According to Foa the hemoglobin is limited to a peripheral layer of granules under which lies homogeneous cell protoplasm, and this view is supported by the occasional escape in pathological conditions of a central achromatic substance, leaving the hemoglobin more or less intact.

Size of Red Cells. *In normal subjects* the average diameter of red cells varies between 7μ and 8μ, depending considerably upon the method employed in preparing the specimen. The following table, compiled by Limbeck, shows the results obtained by different writers:

	Limits.	Average.
Welcker	4.5–9.5μ	7.2–8.1μ
Valentin	....	7.0
Malinin	....	7.7
Hayem	6.0–8.8	7.5
Malassez	....	7.6
Laache	6.0–9.0	8.5
Bizzozero	....	7.0–7.15
Gram	6.7–9.3	7.7–8.0

From 75 to 90 per cent. of the cells fall within average diameters. *Sex* has no influence upon the size of red cells, and after infancy the diameters remain constant. In normal infant's blood the variations in the size of the cells are considerable, 3.3μ to 10.3μ (Hayem).

In pathological conditions variations in the size of red cells are one of the most important of morphological features. In general, variations in the size of the cells indicate a severe and chronic anemia, while in mild and in very acute anemias the diameters are usually little altered.

Anisocytosis is a term used to indicate variations in the size of red cells without the deforming changes in shape involved in poikilocytosis.

Microcytes are cells distinctly under the normal size, and may show a diameter of 4μ to 1μ.

Several influences appear to be concerned in the formation of microcytes. It is probable that cells poor in Hb, lying in plasma of high gravity and osmotic tension, may be reduced in size from the direct loss of substance and the concentrating influence of a fluid of high osmotic tension. Many microcytes probably result from the splitting of portions of degenerating and ameboid red cells, as in pernicious anemia. The occasional occurrence of nucleated red cells of small or minute size indicates that some microcytes arise by division of undersized mother-cells. This mode of origin is probably limited to pernicious anemia.

The clinical significance of the presence of undersized cells or distinct microcytes is rather indefinite. In some cases of chlorosis the cells appear to be uniformly smaller than normal, but this character is not invariable. In some cases of chronic anemia of pernicious grade the majority of cells are undersized and deficient in Hb. In primary pernicious anemia microcytes with increased or diminished Hb are constantly present. Tallquist believes that the presence of many microcytes is an indication of rapid destruction of blood.

Megalocytes are cells distinctly larger than normal. They usually vary between 10μ and 20μ in diameter. Most of these cells form from the division of the large nucleated red cells which appear in red marrow in severe chronic and especially in pernicious anemia. In addition to this mode of origin it is probable that many slightly enlarged cells and megalocytes are produced by the swelling which, under the laws of osmosis, results when red cells lie in plasma of low gravity and low osmotic tension. Megalocytes may show an excess or a deficiency of Hb, and frequently exhibit a brownish stain after eosin.

The presence of megalocytes indicates an anemia of severe grade and usually of rather long standing. Their appearance denotes regenerative activity on the part of the blood (Tallquist). Some authorities arbitrarily suggest that when megalocytes form more than 10 per cent. of the cells the case should be classed as pernicious anemia. In secondary pernicious anemia the megalocytes are frequently deficient in Hb, while in primary pernicious anemia they usually contain an excess of this principle.

Nucleated Red Blood Cells. While the presence of nucleated red cells in the blood at any period of extra-uterine life may be considered pathological, it must be admitted that prolonged search in the blood of infants shortly after birth will usually disclose the presence of a few of these cells.

Normoblasts are nucleated red cells of normal size and character (7μ to 9μ). They usually show a normal or slightly increased quantity of hemoglobin, fail to show a biconcave form, do not cohere in rouleaux, and exhibit central densely staining spheroidal nuclei occupying one-third of the diameter of the cell. Much less frequently the nucleus is subdivided into two or three lobes, more or less closely connected, while mitotic figures in normoblasts have several times been described (Luzet, Troje, Askanazy[1]).

Normoblasts are most commonly seen, in very variable numbers, in the milder forms of anemia, chlorosis, acute anemia from hemorrhage, early leukemia, etc. They may be very abundant in the anemia of children, especially in v. Jaksch's type. In severe anemia they are usually associated with larger nucleated red cells. In children any severe disturbance of the circulation, especially if accompanied by affection of the bone-marrow, may cause the appearance in the blood of a few normoblasts. Severe leucocytosis in children and even in adults may draw a few normoblasts into the circulation. In anemic subjects antemortem leucocytosis may be of this character. In chlorosis a periodical increase of normoblasts, lasting four to six days, repeated at intervals, apparently favored by rest in bed and successful medication, and accompanied by a marked increase in red cells, has been described by v. Noorden under the term "blood crisis." This phenomenon chiefly has given rise to the belief that the appearance of normoblasts is of favorable import in anemia. This opinion may be accepted, not because the normoblasts appear in the circulation, but because their appearance indicates active reproduction and discharge of red disks from the marrow, and demonstrates that the type of blood formation is physiological.

Megaloblasts are nucleated red cells of larger than normal dimensions (10μ to 20μ). When exceeding 20μ in diameter, they are usually called *gigantoblasts.* They are usually circular in outline, but are readily deformed in spreading. Megaloblasts may show a deficiency of hemoglobin, but usually contain an excess, in which case they are apt to stain brownish with eosin (polychromatophilia). The nuclei of megaloblasts show a great variety of forms. They may be found in: (1) the ordinary *vesicular* form of the resting stage, with intranuclear network and nodal thickenings, but without nucleoli; or they may be (2) *pyknomorphous*, consisting of one or several densely staining lobes, either distinctly separate or grouped in irregular rosette form; or (3) they may exhibit phases of normal or pathological mitosis, with irregular wreaths or two or three unequal asters; or (4) they may show many stages of karyorrhexis, with fragmentation, vacuolation, fading of segments of nuclei, as well as minute subdivision into fine basic staining particles widely scattered in the cell. (See Plate VI.)

The significance of the presence of megaloblasts in the blood depends on the conditions with which they are associated.

Megaloblasts are seen in smears of the marrow of many young infants, and when seen in the circulation of such subjects in small numbers and with a majority of small nucleated cells, they have the same significance as normoblasts. In cases of severe anemia of adults a few nucleated red cells of moderately large dimensions are sometimes seen with a larger number of normoblasts, when no special significance can be attached to their discovery.

When the majority of a considerable number of nucleated red cells are megaloblasts, especially if gigantoblasts are present and unequal mitotic figures are observed, the diagnosis of primary pernicious anemia is justified. The presence of a few megaloblasts alone is usually found only in primary pernicious anemia, but may occur in pernicious secondary anemia. Possibly the discovery of a single gigantoblast may warrant the diagnosis of primary pernicious anemia.

In general, the appearance of megaloblasts in the blood indicates that a pathological type of blood formation has been established in at least a part of the red marrow. Whether this change represents a reversion to the embryonal type of blood formation, as suggested by Cohnheim, cannot yet be determined, and will be considered later. Probably the low osmotic tension of the watery plasma may be responsible for some of the increased size of the nucleated red cells of pernicious anemia.

Pathological Changes in Red Blood Cells.

Simple loss of Hb is one of the commonest changes occurring in red cells, this being the chief cellular lesion in chlorosis and mild secondary anemia. When deficient in Hb the cell exhibits an enlarged central area of variable outline. In extreme cases the cell may be reduced to a very thin ring of Hb, inclosing a wide and perfectly transparent central area. In dry specimens, such cells when thinly spread and rapidly dried may fail to exhibit a marked central clear area, but the whole cell shows a uniform pallor. Occasionally the central clear area is irregular, owing either to ameboid changes in the red cell or to the unequal loss of Hb in different parts of the cell, or, frequently, to artificial influences. Thus may arise minute clefts and the heaping of remnants of Hb in the centre of the cell. (See Plate VII.)

Poikilocytosis is a term applied to the appearance in blood of misshapen red cells. Probably the chief mode of origin of poikilocytes is through the effects of ameboid motion of a portion or the whole of a cell, which is one of the phenomena occurring in degenerating red cells. A great variety of shrunken and misshapen cells are produced *artificially* in dried specimens of anemic blood. The true poikilocyte is usually a pear-shaped cell with a short globular projection at one or more poles. Extremely irregular cells usually result from shrinkage or trauma. Very small cells may be formed by the separation of fragments of poikilocytes (Ehrlich).

Poikilocytosis is an indication of a severe anemia with degenerative changes in the red cells.

Ameboid motion of entire cells or of fragments of cells, or of central colorless masses in necrobiotic cells, may be observed, in slight degree, in many severe anemias. This phenomenon, which is more commonly observed in the blood of the low vertebrates, is probably responsible for the production of many poikilocytes and for the subdivision of red cells, but is otherwise lacking in special significance (cf. Askanazy).

Variations in size of red cells is an important feature of degenerative processes which is considered elsewhere (p. 97).

In cases of *acute poisoning* in man and animals, a considerable variety of degenerative changes in red cells has been described by many writers, but these do not yet admit of accurate classification. Among them may be mentioned: (1) the *hemoglobinemic degeneration* of Ehrlich, which consists in the appearance of droplets of Hb within the cell; (2) the *subdivision of the cell* with formation of microcytes, as seen in some forms of poisoning (Litten) and after severe burns; (3) the *specific reaction of diabetic blood* described by Bremer; and (4) the *changes in color* to be noted after poisoning by hydrochloric acid, hydrocyanic acid, chlorate of potash, and some coal-tar products.

The *malarial parasite* produces a peculiar series of degenerative changes in the red cell which will be considered later.

Anemic or Polychromatophilic Degeneration. Polychromasia. Polychromatophilia. Under the term polychromatophilia two apparently distinct conditions have been described.

The term is applied by Ehrlich[1] to the appearance, especially in cases of chronic anemia, of cells which, after hematoxylon and eosin, instead of staining light red take a bluish red or violet tinge, or, in extreme examples, even take a deep blue stain. These various abnormal tints are referred by Maragliano, Castellino, and Ehrlich, to a progressive *coagulation-necrosis* of the cell which thereby loses its power to retain Hb, and, gradually yielding this element to the plasma, loses also its normal affinity for acid dyes. This opinion is based on the following grounds: (1) the appearance of cells showing an advanced stage of the change, in which the frequent breaks in contour "convince every practised eye that the cell is approaching solution;" (2) by the abundance of such cells in starving animals where new formation of red cells is not to be expected; (3) by their abundant presence within twenty-three hours after hemorrhage, when normoblasts are only exceptionally seen; and (4) by the constant polychromatophilic tendency of megaloblasts as compared with normoblasts.

Gabritschewsky, Smith, Askanazy,[2] Engel, Dunin, and many others, believe that polychromatophilic disks are not degenerating, *but, on the contrary, are the youngest red cells of the blood.* This view is supported by the abundance of such cells shortly after severe hemorrhages and in many other conditions where red cells are being rapidly formed. Askanazy found polychromatophilia of all the nucleated red cells in the marrow of a rib just after its excision and before degenerative changes could have set in, and states that a large proportion of cells in the liver of the seven months' fetus are polychromatophilic. Walker also found as high as 34.75 per cent. of basophilous red cells in the blood of a fetal puppy, while in the marrow of various healthy domestic animals they varied from 12 to 62 per cent.

Engel has shown that there is a physiological polychromasia belonging to a portion of the red cells of the embryo, and Ehrlich[2] accepts this fact without yielding his opinion that, in the adult, polychromasia is a sign of degeneration. According to Engel, polychromatic cells appear when rapid new formation of red corpuscles is demanded and when there is not time for the complete shrinkage of nucleus and development of orthochromatic cells.

The bearing of the first studies of Maragliano and Castellino is here of importance. These observers found that normal blood in carefully prepared fresh specimens begins to show distinct alterations of the red cells after standing ten to twelve hours. These consist in widening of the central colorless area, appearance of ameboid activity in this area, formation of poikilocytes and development of a basic staining quality in a part or the whole of the cell. In various pathological conditions they found these changes present immediately after shedding, and the more rapid their appearance the graver was the general condition of the patient. The authors believe that these changes may occur in the circulating blood; that they are referable to decreased stability of cells and increased globulicidal activity of plasma; and that they afford valuable clinical evidence of destruction of blood. They depict cells which show the polychromatophilic tendency of Ehrlich, and *other cells in which there is the formation of a central mass which stains bluish by methylene blue** and which has been shown to herald the extrusion of a portion of the red cell in the form of blood plates. (See Plate II., Fig. 2.)

The processes represented by these two kinds of altered cells may be related, but are certainly not identical, and since the chief evidence in favor of the degenerative nature of Ehrlich's polychromatophilic cells is found in the studies of Maragliano, it would seem that further investigation is necessary before one can accept the view that such cells are necrotic.

Finally, it should be mentioned that Sherrington refers the brownish staining reaction frequently seen in red cells in severe anemia to *incomplete oxidation of Hb.*

To summarize the evidence in this field it appears that two different changes in red cells have been included by different authors under the term polychromasia: (1) A diffuse brownish or polychromatic staining tendency in red cells (polychromasia of Gabritschewsky—Plate VII., Fig. 5) occurs in various forms of anemia, abundantly in normal marrow, and especially in fetal blood and marrow It is probably referable to simple deficiency in quantity or change in quality of hemoglobin, and, while not essentially a degenerative process, is frequently seen in combination with granular degeneration of the red cells, with which its significance must then be regarded as identical. (2) The appearance in red cells of large or small basic staining areas (polychromasia of Maragliano—Plate VI., Fig. 6) occurs in severe anemia and toxemia, has no relation to the other type of polychromasia, but is related to the granular degeneration of Grawitz. It is an evidence of degeneration of the red cells.

Granular Degeneration of Red Cells. Punctate Basophilia of Red Cells. The attention of pathologists has long been attracted by the presence of bluish staining granules and masses in red cells, and they have repeatedly been interpreted as remnants of a nucleus. In specimens stained by the Nocht-Romanowsky method these masses usually give the reaction of chromatin. Lately the formation and significance of these bodies have been fully considered by Maximow, who calls them "nucleoids" and whose conclusions regarding their nuclear character can hardly be doubted.

In 1893 Askanazy[1] described the appearance of many fine basophilic granules in the bodies of nucleated red cells in pernicious anemia, regarding them as evidence of karyorrhexis. In 1894 Schumann saw such cells in the anemia of *bothriocephalus*, and in 1896 Lazarus described them in twenty cases of pernicious anemia, while failing to find them in other diseases. Later they were described in pernicious anemia and variously interpreted by Klein, Zenoni,[1] Lenoble, and Grawitz.[1] Very similar changes have been described in secondary anemia, normal blood, in bird's blood, and in that of the human embryo by Askanazy, Gabritschewsky, Klein, Pappenheim, and Engel.[2] Plehn found them abundantly in the blood of malarious subjects and regarded them as remnants of the parasite.

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* Ehrlich warns against the use of methylene blue in demonstrating polychromatophilia, using only the triacid mixture, or hematoxylon and eosin (Die Anemie, p. 34).

Grawitz has recently described the appearance and studied the nature and occurrence of such cells in human blood, concluding that they represent a specific form of degeneration. He did not find any evidence of increased karyolysis in the normoblasts of marrow when degenerating cells were found in the blood, except in very severe anemia. The exact origin of the bluish staining granules he is unable to explain, but thinks they have no connection with a process of karyolysis. In pernicious anemia and leukemia Grawitz could always find many cells in granular degeneration, but in eleven cases of severe chlorosis they were missing. In carcinoma they were present when the tumor was in such a situation "as to favor the absorption of toxic substances." Thus they were present in ten cases of gastric and esophageal cancer, but absent in two cases of uterine carcinoma. They were not found in numerous cases of tuberculosis and syphilis. In lead poisoning they have been found very abundantly, and often before any subjective symptoms can be detected, by Grawitz, Behrendt, Sabrazes, White and Pepper, and others.

Bloch noted that they are sometimes entirely wanting in fatal pernicious anemia, and he found none in a fatal case of hemophilia. They were more abundant in his cases of carcinoma than in sarcoma, and were absent in cirrhosis and pseudoleukemia, and in one of three cases of plumbism. Burckhardt also saw several cases of lead colic without degenerating red cells. Lowenthal produced them by injection of salts of tin, and they have been seen to result from the action of copper, potassium chlorate, mercuric bichloride, pyrodin, and phenylhydrazin. Stengel, White, and Pepper found them in a great variety of anemias and toxemias, but invariably only in lead poisoning. They demonstrated the granules in the fresh blood by injecting methylene blue into the jugular vein of a leaded dog.

The writer has frequently noted these changes in red cells in pernicious anemia, and is convinced that in megaloblasts they often result from karyorrhexis, having seen many transitional stages of the fragmentation and fine subdivision of these nuclei. In the hydremic blood of malarial cachexia they are extremely common. In some cases, after overstaining in methylene blue, nearly every red cell shows such bluish points, sometimes connected with fine threads.

The origin and significance of these basic staining granules in red cells has been the subject of much controversy.

Ullman regards them as artefacts, and while this view cannot be accepted for all cases their variability in plumbism and other conditions, and the capricious behavior of the red cells in hydremic blood suggests that great care in fixation and staining is demanded in their study.

Some observations favor the belief that granular degeneration is a character exhibited principally by immature red cells, and does not necessarily represent a regressive progress. Bloch found them very commonly in polychromatic cells, and abundantly in the red cells of fetal mice when the mother's red cells were normal. They occur abundantly in healthy animals after hemorrhage, and in disease marked by increased red-cell formation (Jawein).

Grawitz especially, and many others, maintain the origin from a regressive and degenerative process affecting previously normal red cells.

The exact nature of the change is also disputed. The majority of observers believe that the granules are reappearing particles of the diffuse nuclear constituent of the red cell.

Grawitz and Bloch deny that they have any relation to karyolysis or to nuclear elements, but hold that they represent a precipitate of the dissolved albuminous constituent of the cell. Grawitz distinguishes rightly between the large fragments of nuclei seen in karyorrhexis of megaloblasts and the fine granules which are found in the true degenerative process, and disposes of the theory that the granules always result from karyorrhexis of the nucleus in the normoblastic stage. Bloch and Lazarus have seen granular degeneration of the protoplasm of mitotic megaloblasts. It is further argued by Bloch that the granules cannot be nucleinic acid because they show no selective affinity for methyl green, and stain blue with Nocht's method, which colors chromatin red. Against these considerations must be urged the fact that the red cell contains diffuse nuclear material from which the granules may arise apart from karyorrhexis, as Maximow has clearly demonstrated, and that Nocht's stain leaves some nuclear elements blue and others red, and may be made to stain these particular granules deep red, as in the blood plates.

The Numbers of Red Blood Cells. It is hardly necessary to point out that the present method of enumerating the red cells in a unit of volume of blood gives an imperfect estimate of their total number in the body. Yet the conclusions that are constantly being drawn from such information could be justified only in case there was a uniform relation between the number of cells per cubic millimetre and their total number in the body. The considerations mentioned in the Introductory Section show that in pathological conditions no such relation uniformly exists. On the other hand, since the total quantity of blood in healthy subjects is properly adapted to each individual, the bulk is of less practical importance than the proportion in the unit of volume, and the enumeration of red cells in tolerably healthy cases, therefore, warrants the same conclusions that could be drawn from the estimation of their total numbers in the body.

The original estimates of Welcker (1854), that in the cubic millimetre of blood there are normally 5,000,000 red cells in men and 4,500,000 in women, have not been seriously disturbed by many subsequent observations by means of Malassez's method. Perhaps the chief contribution of later observers using Thoma's instrument has been the proof that the numbers are more apt to exceed rather than fall below these averages, especially in men, a fact that has become more certain from the more careful estimates of the last decade. Thus the average obtained by Helling was 5,910,000; by Frederickson, 5,072,000; Zaslein, 5,010,000; Neubert, 5,603,000; Graber, 5,081,000; Stierlin, 5,752,000; Reinicke, 5,209,667; Andriezen, 6,000,000; Hayem, 5,500,000.

The variations in the number of red cells referable to physiological and accidental causes are so numerous and distinct as to require their consideration in detail.

1. **Time of Day.** The variations found by some observers at different hours of the day may probably, as Limbeck suggests, be referred to the influence of digestion and exercise. Reinert found a tendency toward diminution of red cells and increase of leucocytes as the day progressed with healthy subjects.

2. **Digestion.** With but few reports to the contrary, the observations of Vierordt and Duperie, Reinert, and Limbeck indicate that within one-half to one hour after the ingestion of a full meal the proportion of red cells begins to diminish, falling usually 250,000 to 750,000 per cubic mm. The numbers diminish for two to four hours, and then gradually approach the normal. The percentage of Hb falls proportionately. These effects are more prominent after a largely fluid meal, and are probably referable to the absorption of fluids with dilution of the blood.

For the same reasons hunger has been found by many observers to cause in animals a considerable increase in red cells (Bidder and Schmidt, Voit, Subotin, Panum, Groll, Hosslin, Raum).

The observations on man by Raum, Senator, Luciani, and Grawitz,[2] do not, however, show that such an increase is either constant or invariable in fasting men. Twenty-four hours' fasting usually suffices to raise the red cells of dogs 400,000 to 500,000, and in captivity they may emaciate from starvation, while the red cells continue above normal (Hosslin); but in man there has been a lack of uniformity in the changes observed, Senator and Luciani finding an irregular increase in the number of red cells in the professional fasters Cetti and Lucci, while Raum and Grawitz[2] found a distinctly anemic condition established in healthy fasting men.

3. **Sex.** The relative anemia of women is not found until menstruation is established at about the fourteenth to sixteenth years. Indeed, Stierlin, who examined the blood of ten boys and ten girls under fifteen years, found a slight and rather uniform difference in both red cells (350,000) and Hb (2.5 per cent.) in favor of female children.

While the figures of Welcker, 500,000, have been generally accepted as representing the difference between the blood of men and that of women, Leichtenstern, from 191 observations, placed this difference at about 1,000,000 cells. Later reports (Ziegler, Stierlin), as well as common clinical experience, would seem to indicate that the difference does not usually amount to 500,000, and that when the number falls below 4,500,000 in women there is some distinctly pathological condition to account for the relative anemia. There are at hand no systematic comparisons of the number of red cells in women at different ages, apart from the immediate effects of menstruation and pregnancy. Stierlin has shown that both red cells and Hb are subject to more extensive physiological variations in women than in men. In specific gravity Dieballa found that with the same red cells and Hb the blood of women averages 0.0025 less than that of men.

Menstruation. Since a hemorrhage which removes less than one-fiftieth to one-twentieth of the total volume of blood fails to noticeably affect the blood (Vierordt), the menstrual flow, normally about 100 to 200 c.c. and extending over a period of some days, usually fails to reduce the red cells. On the contrary, both red cells and Hb have frequently been found slightly increased after normal menstruation (Vierordt, Hayem, Duperie, Scherpf, Reinl, Reinert). This result has been referred to stimulation of blood-forming organs. A slight leucocytosis has also been noted by Hayem, Moleschott, and Reinert. The clinical rule that normal menstruation in healthy subjects fails to induce anemia but may do so when associated with other causes of anemia, is borne out by Reinert's observations on a chlorotic girl. In this patient a moderate flow re-established through the use of iron failed to lower the blood count, but at two succeeding periods more profuse discharge was followed by a reduction in seven days of 700,000 cells. The changes which occur under the most favorable circumstances are probably illustrated by the results of Sfameni, who examined the blood of six healthy women before, during, and after menstruation. He found that normal menstruation reduces the Hb 4 to 15 per cent. and the red cells 220,543. During the menstrual period the leucocytes are usually slightly increased, average 330, and the decrease of red cells is less marked (122,443). In the intermenstrual period the red cells slowly increase, reaching a maximum three days before the succeeding flow.

In spite of the above largely negative results, it can hardly be doubted that a slightly disordered menstruation is a most important factor in the etiology of chlorosis.

Pregnancy. After passing through many phases of conjecture, opinions regarding the condition of the blood in pregnancy are now based on numerous observations extended and summarized by Schroeder, which show that pregnancy in itself has little influence on the Hb or red cells of the blood, and that if anemia exists it is to be referred to unhygienic conditions. Postpartum anemia should not continue longer than ten to fourteen days, and may be succeeded by a slight increase of red cells above the normal.

PLETHORA. POLYCYTHEMIA.

It has already been shown that there are good grounds for accepting the old belief that there are considerable physiological and pathological variations in the total quantity of blood in the body, and it remains to consider here those factors which directly affect the proportion of red cells.

Polycythemia may arise either from an increase in the number of red cells or from a decrease in the volume of the plasma. Practically it can hardly be said that a true polycythemia from the first-named cause is ever observed, as the blood-forming organs are never known to produce an excess of red cells, and the effects of transfusion of blood are very transitory. Polycythemia from decrease in the volume of plasma, however, is very frequently encountered, and among its causes may be mentioned diarrhea, dysentery, cholera

(Schmidt, Hayem), or any disease attended with excessive watery exudate; conditions marked by insufficient aeration of the blood and venous stasis, as endocarditis, emphysema, asphyxia, and in the peculiar permanent polycythemia observed at high altitudes; the administration in therapeutic or poisonous doses of various drugs, lymphagogues (Grawitz[4]), phosphorus (v. Jaksch), pilocarpin, eserin, etc. (Hamburger), and cold baths. Various other influences leading to *local polycythemia* have been mentioned in the Introductory Section.

The danger of overlooking the true cause of polycythemia is well illustrated in Timofjewsky's experiments, when after the intravenous injection of pus an increase of red cells of 1,500,000 to 2,000,000 was found to result from concealed exudates from mucous and serous membranes.

The Polycythemia of the Newborn. The marked relative polycythemia of the blood of newborn infants has been constantly attested. The following averages have been observed:

Hayem	5,368,000
Sorensen	5,665,000
Otto	6,165,000
Helot	5,531,000
Schiff	5,825,000
Bidone	6,500,000

While the averages of considerable numbers of cases reported by the above authors are rather uniform, very marked variations have been noted, referable to changes at different periods of the day, and to individual peculiarities. Hayem and Helot found that too early ligature of the cord might cause a reduction of 500,000 to 1,000,000 cells. During the first weeks the polycythemia, which is usually highest before the first nursing, gradually disappears at the rate of about 250,000 cells weekly, but while the average fall of a considerable number of cases is very regular, there are marked daily variations in individuals of from 500,000 to 1,000,000 cells (Schiff). No definite relation between the changes in body weight and the number of red cells has been traced, but a marked reduction usually follows each nursing.

The cause of the polycythemia of the newborn is doubtless to be found in a temporary concentration of the blood in which a number of factors are concerned. The loss of water during the first hours of respiration has been regarded as an important influence (Preyer), but can hardly account for the condition of the blood in the first hours after birth. The writer finds that the polycythemia bears a rather close relation to the degree of cyanosis exhibited by the expressed blood drop, and believes that the concentration of blood is principally referable to a state of relative stasis which is then established in the peripheral capillaries.

The Polycythemia of High Altitudes. The observations of Bert in 1882 that the blood of animals from the Bolivian plateaus showed much increased capacity to absorb oxygen, led Viault to examine his own blood while sojourning in the Andes and to find that at an elevation of 4392 metres the red cells had increased from 5,000,000 to 8,000,000, many of them being undersized. In the blood of animals he found a similar polycythemia but no increase in oxygen.

These observations were verified by Egger, Koppe, Wolff, and many others, who found that the increase begins almost immediately upon the change in altitude, amounting at times to 1,000,000 cells in twenty-four hours, while Campbell and Hoagland calculated that there is an increase of 50,000 cells for every 1000 feet of elevation. The increase reaches its limit in about two weeks, after which the numbers tend slowly to diminish. On returning to sea level the polycythemia promptly disappears, even more rapidly than it is established (Mercier). The percentage of Hb appears to be less affected, and the volume of red cells not at all. Egger found no change in the dry residue of the blood of two rabbits, but Grawitz found the residue distinctly increased in a rabbit which had been confined in a rarefied atmosphere. The blood of anemic and phthisical patients is less markedly and permanently affected than that of healthy individuals.

The significance of these changes has not been fully explained, but it seems most probable that the polycythemia is an effect of concentration of the blood, brought about by lowered blood pressure, and tending to readjust the respiratory function to the smaller proportion of oxygen in the rarefied atmosphere. That the state of the blood is one of simple concentration is, however, rendered uncertain by the low percentage volume of red cells. Egger and Koppe maintain that there is a formation of new red cells, finding a sudden increase of small cells soon after the ascent, while at the end of the period there are four to eleven small cells to one large one. These microcytes, however, cannot figure as evidence of new formation of cells. Normoblasts ought to be present if, as Grawitz says,[3] about one litre of new blood has been produced in twenty-four hours, while the polycythemia promptly subsides at sea level without yielding signs of blood destruction. The possibility that the cells are concentrated in the capillaries is denied by Egger, who found a similar increase in the large internal arteries of rabbits, and claimed by Campbell and Hoagland, among others, who found the red cells diminished in the blood of the mesentery while increased in the ear. The suggestion of Fick that the red cells may enjoy a lengthened existence in the circulation does not commend itself.

Several observations, recently reviewed by Starcke, indicate that the results obtained by the hematocytometer vary to some extent with the temperature and barometric pressure, and Zeiss constructs a special counting-chamber, allowing a free circulation of air beneath the cover-glass for use in high altitudes. It has not, however, been found possible to refer the polycythemia of high altitudes to factors of this sort.

Residence at the *seacoast* is frequently followed by increase in the number of red cells as a part of improvement in general health.

In the *tropics* most Europeans becomes more or less anemic until fully inured to the various unhygienic influences obtaining there.

The Polycythemia of Diarrheal Diseases. The concentration of the blood which results from depletion of fluids through the intestine is seen to a slight extent in severe simple diarrhea, more distinctly in acute dysentery and at its height in cholera.

An increase of two millions in the number of red cells has been observed by Hay within two hours after the administration of 21 grm. of Glauber's salts in concentrated solution, and Brouardel demonstrated less clearly a marked increase after purgation by croton oil and jalap. When given in a dilute solution the effects of the salines are, of course, less evident. Clinically, a moderate polycythemia has been observed after severe diarrhea or vomiting, but the effects are usually transient.

In dysentery, polycythemia may be expected only when the diarrhea is acute and the quantity of blood lost is slight. Usually an anemia is established by direct loss of blood.

In typhoid fever the progress of the anemia is often greatly obscured by the concentration of blood in cases marked by severe diarrhea. In cholera the conditions are especially favorable for watery depletion of the blood and tissues, and remarkably high counts have been recorded in the late stages of this disease.

Polycythemia of Endocarditis, Venous Stasis, etc. Very numerous observations have shown that at some stages of endocarditis a marked polycythemia is established, and largely through the studies of Oertel and of Grawitz it has been shown that this concentration is found in conditions marked by chronic stasis, cyanosis, edema, and is more marked in the capillaries than in the veins or arteries. The same condition is seen in chronic stasis from other causes (Peiper, Grawitz[5]).

Phosphorus Poisoning. In cases of phosphorus poisoning of moderate intensity, but fatal, Taussig observed an increase in red cells to 8,500,000, v. Jaksch an increase from 4,300,000 to 8,250,000 and Limbeck an increase to nearly 8,000,000. The fact that the polycythemia does not appear until the toxic symptoms are marked, and usually subsides with them about the fourth or fifth day, indicates that it results largely from depletion of the blood through the vomitus. v. Jaksch found no simultaneous increase in the proportion of albumins. The leucocytes are usually diminished, sometimes slightly increased, and the Hb-index is lowered.

Illuminating Gas Poisoning. Munzer and Palma, and Limbeck have observed moderate polycythemia in three cases (5,700,000, 6,630,000, 5,700,000), with slight leucocytosis, 13,300 in one case. The condition must be referred to venous stasis.

Polycythemia after Cold Baths. An increase of red cells of 500,000 to 1,500,000 has been observed by many writers to occur after the application of cold baths (Toenissen, Winternitz, Knopfelmacher, Thayer). The change is observed immediately after the bath, and continues after cyanosis has disappeared and while the patient is shivering, but soon disappears thereafter. It is often missing after fifteen to twenty minutes. The leucocytes are also usually increased (15,000 to 20,000). The condition is probably referable to concentration of the blood due to stimulation of the vasoconstrictors, and is probably more marked, if not exclusively present, in the peripheral capillaries. Yet some have suggested that a new formation of cells may be indicated by the change. It may be noted here that warm baths have a contrary effect.

Effects of Surgical Procedures upon the Blood. Anesthesia.

Several painstaking studies have been contributed in the last few years in the endeavor to determine what effects upon the blood are produced by the administration of ether and by the procedures required in a surgical operation.

(*a*) **Effects of Ether.** Chadbourne counted the leucocytes before and after etherization, but before operation, in twenty cases, most of whom exhibited leucocytosis from some inflammatory condition. He found an increase of leucocytes in each instance, often slight, but sometimes well marked, averaging 37 per cent. The increase was most marked in the early stages of anesthesia. Differential counts in five cases showed very slight variations in the proportions of the different types of cells.

Of 50 cases examined under similar conditions, Cabot, Blake, and Hubbard found the leucocytes decreased in 7, increased less than 2000 in 30, more than 2000 in 13, and more than 10,000 in 1 case. The discrepancy in the above results may be explained by differences in the methods of estimating the leucocytes and of administering the ether and in the character of the cases under observation, and it may only be said that etherization of human subjects requiring operation usually increases the leucocytes to a slight degree (2000), but sometimes very markedly, or, again, may not increase them at all or even cause considerable diminution. More uniform results may perhaps be obtainable from experiments upon animals. Oliver etherized eight rabbits for one hour, finding the red cells diminished on the average 0.03 per cent., far within the limit of error in calculation. In three rabbits the writer found no appreciable difference in the leucocytes of the jugular vein before and after full etherization.

(*b*) **Effects of Ether and Operation.** Most of the studies in this field concern the changes in the blood after operation.

Da Costa and Kalteyer, in fifty cases, found the red cells increased 149,000 (average), Hb reduced 3 per cent., and leucocytes increased 4586. They found reason to believe that the preparatory treatment of the patient by fasting and purging concentrates the blood to some extent.

Frazier and Holloway, in forty cases, found very slight changes in the red cells and Hb, as the following table shows:

Before operation.	Three hours after.	Twenty-four hours after.	Forty-eight hours after.
Hb., 81.8 per cent.	82.9	81.6	79.3
Red cells, 5.49 mil. . . .	5.58	5.53	5.27

The leucocytes invariably increased, sometimes very markedly, in seven cases of malignant tumors from 11,000 to 24,777; in twelve inflammatory cases, from 12,498 to 20,414, and in twenty-one non-inflammatory cases from 9748 to 20,053.

Very similar results in the study of leucocytosis after operation have been reported by v. Lerber, White, Cabot, Blake, and Hubbard, but in five of forty-seven cases of the last-named observers there was a decrease of white cells.

Frazier endeavors to analyze results with regard to many factors concerned with the operation. He concludes that the leucocytosis increases in general with the extent of the operation, is not affected by fever, nor by the administration of ordinary drugs, by vomiting, sweating, purging, or pain. The usual dressings have very slight influence. Hemorrhage may cause considerable increase. Imperfect drainage of the wound, with collections of fluids and infection of the wound, always cause increase of leucocytes. In uncomplicated cases the leucocytes returned to the normal in about three and one-half days in Frazier's cases; in thirty-six hours in Cabot's; in twenty-four hours in Bloodgood's, and not until five days in White's celiotomies. In many cases the fall should be progressive after twenty-four hours, otherwise a complication may be suspected.

The results of the above cases are of importance in emphasizing the great variety of factors which must be considered in the interpretation of blood analysis and in the conclusion, which seems to be unanimous with the above writers, that complete routine examination of the blood is a necessary feature of the correct management of surgical cases.

Influence of Therapeutic Measures on the Red Cells.

Iron. The remarkable effect of this agent upon the blood in suitable cases of anemia is seen in a rapid increase in the number of red cells and in a later but rather more uniform increase of Hb. According to Hayem the effects of iron in chlorosis may be divided into two periods, in the first of which the increase in red cells outstrips that in Hb until a normal number is reached, while in the second period the Hb of the new red cells is gradually brought to the normal. The appearance in such cases of many small red cells deficient in Hb has been commonly noted, but Stifler finds they are not equally abundant at all periods of the regeneration of the blood, that the increase of cells occurs periodically, and that at the height of the periods many pale cells are found which increase in Hb-content while the numbers of cells remain nearly constant.

Again, Willcox finds a difference in the effects of small and of large doses of iron. When small doses are used the red cells outstrip the Hb, but with large doses both increase in equal proportion. Laache also has observed equal progress in cells and Hb in cases of chlorosis. It would seem, therefore, that the regeneration of the blood under iron may follow any one of the above three courses, depending upon various circumstances which cannot here be further discussed.

Hayem and Reinert have called attention to the tendency of the red cells to diminish slightly after reaching a normal number until the restoration of Hb becomes complete. Of the two Reinert believes that the progress of the Hb is the more uniform. Limbeck finds that the red cells usually diminish for some days when the administration of iron is begun.

Indications for the Use of Iron. The specific effects of this drug being centred primarily on the Hb, the chief indication for its use and the best results are obtained in cases of pure chlorosis with marked loss of Hb and moderate reduction in cells. In secondary anemia its curative action is less certain. *With the appearance of larger cells with normal or increased Hb the use of iron becomes much less effective*, and when the Hb-index is above normal it seems to be entirely valueless.

A large part of the effects attributed to iron may be obtained solely by the improved hygienic and dietetic conditions which are usually enjoyed during its administration. The influence of rest in bed, selected diet, baths, oxygen, massage, etc., has long been recognized as a very powerful adjunct in the treatment of anemia. But numerous observations go to show that while the numbers of cells may be greatly increased by these means, yet the complete restoration of Hb in severe chlorosis is seldom as complete or rapid as when iron is freely given, so that a specific influence of this drug must be admitted. This fact is clearly indicated in Aporti's experiments on animals subjected to repeated bleedings, in which recovery was greatly retarded on iron-free diet and markedly and promptly facilitated by injections of iron.

Of the great variety of preparations employed at various times, probably the more irritant preparations in large doses are still acknowledged to yield the best results when well borne by the stomach.

The curative action of iron in anemia is still largely unexplained, but the theory of Binz appears to remain most rational. Binz holds that in chlorosis, as a result of prolonged malnutrition, the absorption of iron from the food becomes deficient, while its excretion through the liver and bile remains undiminished, and the blood becomes impoverished in this principle. By administering iron in large doses it is more readily absorbed and the deficiency is overcome.

Bunge's theory, based on Sir Andrew Clark's belief that chlorosis is an auto-intoxication of intestinal origin, assumes that the iron administered is not absorbed in increased quantity but exerts its curative influence by neutralizing toxic agents in the intestines. These assumptions have been largely disproved by v. Noorden, Rethers, and Morner, who have demonstrated that abnormal putrefactive processes in the intestine are not usually present in chlorosis, and by the demonstration that increased ingestion of inorganic iron *is* followed by its increased absorption principally or exclusively in the duodenum (Kunkel, Woltering, Hall, McCallum, Hochaus and Quincke, Cloetta, Hoffman). After intravenous injections of iron Jacobi found that 10 per cent. promptly appeared in the excreta, 50 per cent. lodged in the liver, the rest in other organs, while the blood was free from excess after three hours. v. Noorden's[2] conclusions seem to embody the results thus far obtained, that while iron in the nucleo-proteid combinations of the food is constantly available in chlorosis, it is either not absorbed or is not appropriated by the blood, whereas when given in large doses it is absorbed in excess and exerts a specific influence upon the bone-marrow. The problem of the nature of this influence remains, of course, untouched.

Arsenic. While iron is contraindicated by the appearance in the blood of megalocytes with increased Hb, under these circumstances arsenic frequently exerts an almost specific effect in increasing the number of red cells and in stimulating the production and more uniform distribution of Hb. Its field is, therefore, chiefly in the severe and chronic anemias, but in the simpler forms the action of iron is often accelerated by combination with arsenic. In chlorosis arsenic is said to increase the red cells much faster than the Hb (Riva), and to stimulate the development of more resistant cells (Bettman). Its therapeutical effects in disease are rendered more obscure by the fact that in health it causes diminution in the number of red cells (Stierlin, Delpeuch). Stockman and Greig claim to have found evidences of increased formation of red cells in the marrow of rabbits after long use of arsenic, yet the number of red cells in the blood remained normal.

Mercury. Numerous observations upon the effects of mercury in syphilis, dating from those of Wilbouchewitch and Keyes, in 1874–76, to those of Lezius and Martin and Hiller, in 1890, have failed to demonstrate that this drug in therapeutical doses has any direct effect upon the red cells. It has been shown, however, that in doses beyond a limit which varies with individuals and circumstances, mercury usually causes a prompt reduction in both cells and Hb, and distinct anemia. It does not appear, from these studies, that the improved condition observed in the blood of syphilitics using mercury is separable from the removal of the virus and the consequent general improvement in health. A reduction in Hb and cells has been frequently observed soon after beginning mercurial treatment or after the drug has been pushed and seems to continue until the system becomes inured or the dose has been reduced to a proper limit, so that the examination of the blood, as shown by Martin and Hiller, may be made a valuable guide in the administration of this remedy. Most of the conflicting results reported may be explained on the grounds that in doses beyond a variable limit, especially at the beginning of treatment, mercury reduces the cells and Hb; that when the original disease is being successfully combated the anemia usually improves; and that apart from its antisyphilitic powers the drug has little effect upon the blood. (See section on Syphilis.)

Lead. In cases of chronic lead poisoning the blood commonly shows a moderate grade of secondary chlorotic anemia. Zinn records an acute case with hemorrhages in which the red cells numbered 4,000,000, leucocytes 3300, Hb 58 per cent., s. g. 1048. More severe cases have been reported with anemia, usually in proportion to length of the disorder, by Malassez, 2,200,000 red cells; by Limbeck, 2,200,000; and by Brochin, 1,300,000. Hayem states that the anemia of chronic plumbism is characterized by its close resemblance to chlorosis, by the rapid decrease in cells while the patient suffers from colic, and by the absence of leucocytosis. Hayem saw no case with increased Hb-index, but Malassez reported megalocytes and megaloblasts as abundantly present in some cases.

This anemia is commonly attributed to gastro-intestinal disturbance, but Hayem referred it to destruction of cells from direct action of lead.

Maragliano demonstrated increased globulicidal activity of the serum in chronic plumbism, and Grawitz has noted considerable degrees of granular degeneration of the red cells.

OLIGOCYTHEMIA.

Hemorrhage causes a peculiar type of impoverishment of the blood in which the oligocythemia is associated with other important changes in the plasma. The progress of events in the circulation which follow a severe loss of blood involves the consideration of many physiological processes. One of the first effects is a lowering of blood pressure, which is followed by an increased flow of lymph and by a rapid transfusion of fluids from the tissues through the capillary walls. The fluid which replaces the lost blood is necessarily of different composition from the plasma, and its addition to the circulation markedly affects the composition of the blood after hemorrhage. The lymph, being rich in salts and poor in albumins, and water from the tissues passing through capillary walls more readily than albumins, the blood when restored to its normal volume is found to be low in albumins, rich in salts, and poor in red cells. After rapid hemorrhages the alkalinity of the blood falls (Zuntz), and its content in sugar increases (Bernard, Mering), this principle being derived from the glycogen of the liver (Schenck), while the coagulability is much increased. The hydremia affects principally the plasma, but Herz found the relative volume of the red cells tripled ten hours after a very severe hemorrhage, indicating that they had absorbed much water.

In small animals (rabbits) the restoration of the volume of blood takes place very rapidly and oligocythemia is observed almost immediately after the hemorrhage, but in larger animals (dog, man) a distinct interval is required before the fluids have replaced the lost blood, and, according to Limbeck, thirty-five to forty minutes may elapse before a distinct reduction in red cells is observed after moderately severe hemorrhages.

The changes in the number of red cells following hemorrhages are somewhat irregular, but from the observations of Huhnerfauth, Lyon, Otto, Koeppe,[2] Viola and Jona, it has been shown that in dogs and man a single large hemorrhage reduces the red cells in proportion considerably less than the effect upon the volume of blood, beginning about one-half hour after the operation, reaching a maximum effect in three to four days, and followed by a restoration to the normal number in nineteen to thirty-four days (Lyon).

In small animals (rabbits) the rate of decrease is more rapid, the lowest point being reached in a few hours, and recovery also following earlier. With slow hemorrhages of large extent (3 to 4 per cent.) the minimum is reached in one to nine days, depending largely upon the size of the animal, and recovery is complete in fourteen to twenty-two days (Huhnerfauth). After lesser hemorrhages (1 to 3 per cent.) full recovery follows in five to fourteen days, while small losses leave no trace after two to five days (Lyon). These periods depend much upon the state of nutrition, and it has been found that full diet, free supply of water, and transfusion of salines greatly accelerate recovery. Ehrlich believes that the continuous reduction in cells is partly referable to the solution of many of them as the plasma becomes more watery.

Dawson's experiments support this belief for cases in which infusion is practised, as he found the red cells to show diminished resistance, while the freezing point of the serum was unaltered. Limbeck and Pick also found that the albumins of the serum are restored within forty-eight hours.

During both phases the Hb falls behind the changes in the cells (Otto, Reinert), owing to the formation of numerous microcytes by the splitting of red cells (Koeppe). Grawitz, however, does not believe that such splitting of red cells can occur. Nucleated red cells of normal or very small size appear within a few hours and in larger numbers after severe hemorrhage, and even after small losses, if sudden. That they are drawn into the circulation mechanically is indicated by their usual absence after slow bleedings of much larger extent (Zenoni[2]).

Changes in the size and shape of the cells are commonly observed, especially after large bleedings. Microcytes and slightly enlarged megalocytes usually appear in two to four days, at the period of greatest oligocythemia (Koeppe.) The extent and duration of these changes depend largely on the grade of anemia established. Polychromatophilia is usually marked within twenty-four hours.

Healthy men recover rapidly from moderately severe hemorrhages and so completely that the red cells may be more numerous than before, from apparent overstimulation of the marrow (Otto, Hall and Eubank). Small and repeated hemorrhages, on the other hand, have led to some of the most severe forms of anemia ever recorded, in which the morphological changes of pernicious anemia are pronounced, but the prevailing feature of the blood is the loss of Hb.

From very extensive observations in Mickulicz's clinic, Bierfreund found that the regeneration of the blood is most rapid in male subjects between twenty and forty years of age, is considerably earlier in males than in females, and in adults than in children or old persons. A loss of 5 per cent. Hb was found to lengthen the period of regeneration two to eight days. The minimum percentage of Hb was reached, after a loss of 10 to 15 per cent., in 3.5 days; after 16 to 20 per cent., in 5.8 days; after 21 to 25 per cent., in 6.5 days; after 26 per cent., in 9.6 days, and in women usually one day later than with men.

It is significant that in Bierfreund's tables there are no averages on patients losing more than 30 per cent. of Hb, indicating that when a surgical operation causes a loss of 30 per cent. of Hb it is usually fatal. Mickulicz attempted to deduce from this fact the rule that no operation should be undertaken unless the patient could stand such a loss, but it is difficult to see any great practical application of such a rule.

BIERFREUND'S TABLE, SHOWING AVERAGE TIME OF FULL REGENERATION OF BLOOD AFTER HEMORRHAGE.

Loss of blood in Hb per cent.	Male.	Female	Age (years).						
			1-10	10-20	20-30	30-40	40-50	50-60	60 -
10-15 . . .	12.7	17.1 days	20	18.2	10.8	10.4	16	17	
16-20 . . .	17.9	23.5	21	19.0	12.5	16.2	21	22.7	28.8
21-25 . . .	20.8	28.5	22.5	21.3	17.6	......	19	28.0	27
26 . . .	27	31.3							

Anemia following Repeated Small Hemorrhages. While very marked changes in the blood following a single large hemorrhage may be rather promptly restored, the regeneration is very greatly retarded after even one repetition of the hemorrhage, and when they are frequently repeated there is soon established a severe form of chronic anemia. It does not appear that this anemia differs greatly from other equally severe secondary forms, but it is usually characterized by marked reduction in the albumin (less of the globulin) of the plasma and serum; by lowering of the dry residue and ash, due to loss of Fe, P, and K, which more than balances the increased Na, and by lowered alkalinity. Besides the actual withdrawal of albumin, repeated hemorrhages seem to have almost a specific effect in increasing fat formation at the expense of albumin both of the blood and tissues (Leube).

The loss of red cells is here an accurate measure of the grade of anemia, and all ordinary forms of their degeneration are observed. In mild cases the loss of Hb is relatively slight, but in some severe cases the Hb percentage and index are very low. On the other hand, there can be no doubt that the state of the blood following repeated small hemorrhages cannot always be distinguished from that of primary pernicious anemia with increased Hb-index. Anemia from hemorrhages is one of the secondary forms most commonly marked by leucocytosis, yet this feature is often not marked and is not infrequently absent.

With man and animals the clinical effects of hemorrhage vary greatly among individuals. Bechamp and Huhnerfauth find that dogs withstand the loss of 3 to 4 per cent. of the body weight, 30 to 40 per cent. of the total volume of blood, if the loss is gradual, but may perish after smaller but more rapid bleedings. As a rule, the smaller the animal and the more rapid the hemorrhage the more severe are its effects. Adult male subjects usually die if losing rapidly 50 per cent. of the blood, but may survive much greater losses from slow bleeding. Children are much more susceptible, and may die after very small hemorrhages, if rapid, while women withstand effects of hemorrhage much better than children or men. The regeneration of the blood, however, is more rapid in men than in women (Bierfreund).

The quantity of blood which may be lost without causing death varies greatly among individual men and animals, as the following table will illustrate:

LIMITS OF HEMORRHAGE FROM WHICH RECOVERY HAS BEEN OBSERVED.

Author.	Animal.	Percentage of body weight lost.	Percentage of red cells remaining.	No. of red cells remaining.
Vierordt	Dog.	....	50	
Hayem	"	4.83–5.55		
Kireeff	"	4.3 –7.3		
Maydel	"	5.48–6.57 average 5.12		
Schram	"	4.58 (not fatal). 5.4 (even chance). 5.44 (always fatal).		
Landerer	"	4.5		
Feis	Rabbit.	3.0		
Andral, Behier . . .	Man.	....	50	
Laache	Woman.	....	82	1,598,000
Hayem	" 2 hemorrhages in 6 days.	....	11	1,415,000

TRANSFUSION OF SALT SOLUTION, SERUM, AND DEFIBRINATED BLOOD.

Siegel and Schram both found no improvement in the regeneration of the blood from the transfusion of salt solution or of serum in animals after bleeding, and while it has since been shown that the regeneration is somewhat more rapid and complete after salt infusion, yet this procedure must be regarded as of more value as a means of saving life than as a stimulant to blood formation. The direct effects on the blood of infusion of defibrinated blood seem to be much more favorable. On the other hand, after the transfusion of blood the above observers and others have found a rapid increase in the number of cells, and Bizzozero reported the same effect after transfusion of defibrinated blood in animals. Quincke was one of the first to note an increase in red cells in pernicious anemia as a result of transfusion of blood, and similar observations have been made by Ziemssen in anemia and scurvy. It would seem from the observations of Bizzozero and of Bareggi that the red cells are quite resistant to the process of defibrination and injection. Of the immediate effects of salt infusion upon the blood of the human subject there are a few reports at hand which indicate that it has considerable influence in lowering the numbers of red cells and increasing the leucocytes.

ORIGIN OF SECONDARY ANEMIA.

The anemia associated with acute and chronic diseases is a result of many factors of varying prominence in different conditions, and requiring consideration in detail.

Diminished Nutrition. Since the digestive powers are largely in abeyance in severe febrile states the loss of the usual supply of albuminous principles must figure to some degree in the impoverishment of the blood. Although the complete withdrawal of food in healthy men leads to a simple "atrophy of the blood," with other tissues, marked by diminution in bulk, polycythemia, etc., but not to anemia (Heidenhain, Voit), it by no means follows that similar results would occur in disease, while distinctly anemic conditions are known to result from improper quality of food (Leichtenstern). Grawitz has found that food deficient in albumin may cause a diminution in the albumin of the plasma, which may be demonstrated within four to eight days, is more marked when the subject is exercising, and later affects also the number of cells.

Diminished Activity of Blood-forming Organs. Simultaneously with the loss of digestive powers it can hardly be doubted that the normal process of red-cell formation must be interrupted in most infectious diseases. This conclusion may be based upon the known effects of toxemia upon cellular processes, or the absence of normal stimulus from absorbed food products, and upon anatomical conditions demonstrated in the bone-marrow. Regarding the last factor it may be said that the leucocytosis of pneumonia is associated with proliferation of myelocytes in the marrow at considerable expense of normoblasts, and in typhoid fever the normal relations in the marrow cords are greatly disturbed by congested, dilated, or even ruptured sinuses.

Increased Consumption of Albumins. The toxic destruction of albumins in fever has been fully demonstrated by Hallervorden and Leube,[2] and their products noted in the increased excretion of urea, of urinary pigment (Jaffe), and potash salts (Salkowski). While the tissue proteids suffer most in this process it is but a step to the source of these principles in the blood. That the albumins of the blood and tissues suffer in some degree in afebrile cachexias is indicated by the results of Müller's studies on metabolism in carcinoma.

Increased Destruction of Red Cells. The increased globulicidal activity of the serum in infectious and septic processes is undoubtedly a most potent agency in the diminution of red cells, and has been demonstrated by Maragliano in carcinoma, pneumonia, typhoid fever, erysipelas, tuberculosis, and many other conditions. The very rapid deglobulization observed in malignant endocarditis and septicemia leaves abundant evidence of this peculiar process also in the pigment deposits found in the viscera. Of the sources of the globulicidal agent little is known, but it is certain that the products of disordered tissue metabolism and bacterial toxins are capable of dissolving red cells when injected into the circulation. Fischer and Adler saw the red cells fall from 6,000,000 to 1,300,000 in seven days after injections of streptococcus cultures into a rabbit; and Grawitz could find only 300,000 red cells in a remarkably rapid case of streptococcus septicemia in an adult woman. Bianchi-Mariotti found a marked loss of Hb in direct proportion to the quantity of culture used after injections of cultures of *B. typhosus*, *cholera*, *anthrax*, etc.

There is abundant evidence to show that in many conditions associated with acute infection and resulting from chronic cachexia the red cells are abnormally fragile. Of such evidence may be mentioned the occurrence of paroxysmal hemoglobinemia on slight exposure to cold, and the rapid development of anemia in many mercurialized subjects.

Loss of Blood by Hemorrhage and Exudation. Perhaps the majority of severe cases of secondary anemia result from the complication of the original disease by single or repeated hemorrhages. Post-typhoid anemia is usually slight and sometimes absent if there have been no bleedings from the intestines, and cancerous cachexia is very promptly aggravated when ulceration and hemorrhage supervene. Much of the anemia in anchylostomiasis is referable to minute hemorrhages.

Of the depleting effect of albuminous and bloody exudates good examples are seen in the acute anemia of exudative nephritis (Dieballa), and in the hemorrhages of the infectious purpuras, tuberculous pleurisy and malignant endocarditis.

Chronic suppuration leads to a peculiar form of cachexia with waxy changes in the viscera, and severe anemia.

By the interaction of these various factors there usually results in infectious diseases and cachexias a certain grade of secondary anemia. Yet, notwithstanding the many contributing causes, acute infectious diseases, *e. g.*, typhoid fever, may run their course with no demonstrable reduction in red cells, and it is a rule of frequent application that associated conditions in these diseases obscure the actual state of the blood and tend to diminish or exaggerate the true degree of anemia.

The polycythemia of cholera is an example of one extreme condition of this sort, partially illustrated also by the slight polycythemia sometimes observed in typhoid fever.

In dropsy the state of the blood accords with the watery condition of the tissues, and anemia is apt to be exaggerated. Grawitz has found that injections of extract of cancerous tumors greatly accelerate the flow of lymph into the circulation and are followed by reduction of red cells.

The morphological changes in the blood of secondary anemias have been rather fully described in an extensive literature upon this subject, and will be detailed later. Of the chemical changes very much less is known, although it can readily be seen that the varying prominence of the above factors probably leads to very different chemical alterations. To what extent constant chemical peculiarities can be established for the secondary anemias of widely different origin, *e. g.*, hemorrhages and syphilis, remains to be decided, but at present there is no evidence to show that any uniform chemical changes distinguish one form of secondary anemia from another (Ehrlich).

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CHAPTER IV.

THE LEUCOCYTES AND LEUCOCYTOSIS.

LEUCOCYTES.

Morphology. *In fresh blood* leucocytes are colorless, rather highly refractive bodies, usually larger than red cells, cohering to one another and to the glass, and exhibiting a highly refractive, compact, or, in the large mononuclear cells, vesicular nucleus. *Granules* are invisible in the normal mononuclear cells of the circulation, but in the others minute opaque (neutrophile) granules are distinctly apparent in the polynuclear cells, and large greenish refractive granules serve to fully distinguish the eosinophile cells. The protoplasm of the mononuclear cells is homogeneous, and refractive in the lymphocytes, transparent in the large mononuclear. *Ameboid motion* begins promptly, especially on a warm stage, in the finely and coarsely granular cells, becomes most active usually after twenty to forty minutes, and may persist for hours. In some later stages of ameboid activity the granules may show extremely active, vibratory, dancing, and swarming movements in cell bodies or processes. These have been regarded as Brownian movements or as indicating structural changes of approaching death of the cell. In many cells, especially in anemic blood, there appear from the first large and small clear spheroidal areas which on staining appear to be divided among watery vacuoles, or granules of fat, glycogen, or other degenerative products.

Varieties of Leucocytes. *In stained specimens* five varieties of leucocytes may be distinguished, viz.:

1. **Lymphocytes.**
2. **Large mononuclear leucocytes.**
3. **Polynuclear neutrophile leucocytes.**
4. **Esoinophile leucocytes.**
5. **Mast-cells.**

1. **Lymphocytes** are classed as (*a*) *small* and (*b*) *large*, the small cells being 5μ to 8μ in diameter, and the large ones 8μ to 10μ. Both sizes exhibit a narrow rim of strongly basophile homogeneous or coarsely reticulated cytoplasm, and compact or coarsely reticulated spheroidal nuclei, which are usually less basophilic than the cytoplasm. With ordinary stains these cells fail to show cytoplasmic granules, although exhibiting nodal thickenings of the cytoreticulum which sometimes resemble granules. After the Nocht-Romanowsky stain all lymphocytes usually show a variable number of large and small violet stained granules, and in some of the larger cells the number of these granules approaches that of the myelocyte. (Plate

III.) The nuclei of lymphocytes usually contain nodal thickenings resembling nucleoli. While the nuclei of lymphocytes are usually spheroidal, there occur in normal blood, and especially in lymphemia, medium-sized, strongly basophilic, hyaline leucocytes with incurved or subdivided nuclei, which must be classed with lymphocytes.

Ehrlich has described these nodal thickenings as nucleoli, although not able to demonstrate in them the specific staining reaction demanded by histologists for the identification of these structures. Jolly was unable to demonstrate true nucleoli in lymphocytes by his method (fixation in Fleming's solution) which succeeded in demonstrating nucleoli in some other leucocytes. Rosin, however, by vital staining with pyronin methyl green demonstrated red nucleoli in green nuclei of lymphocytes, and regards this reaction as specific of the lymphocyte class.

Some authors distinguish two types of lymphocytes, one of which, the *myeloblast*, they believe is an undifferentiated cell and the parent of all blood cells, while the other is a specialized cell derived from the other and retaining permanently the characters of the lymphocytes. (See Chapter V.)

Ameboid motion in lymphocytes was first described by Schultze, the inventor of the warm stage, and has long been accepted by pathologists as a fact. Ehrlich, however, reasoning from the difference in chemotactic sensibility of neutrophile cells and lymphocytes, concluded that the latter possessed no ameboid properties, and his teaching has dominated current opinion for some years. Recently Wolff, Hirschfeld,[3] and Almquist have described anew ameboid movements and the locomotion of lymphocytes in normal blood and in leukemia. They are best demonstrated by examining blood on a warm stage on Deejen's agar-salt medium. Usually the large lymphocytes and hyaline cells have been described as showing the most active changes, but in malarial blood the writer has followed extensive ameboid changes in lymphocytes possessing little cytoplasm. (Plate III.)

2. **Large mononuclear leucocytes** may be only slightly larger than lymphocytes, but many of them are the largest cells seen in normal blood. Their protoplasm is *slightly basophile* and *very finely reticular*, with nodal thickenings often resembling basic granules, while other cytoplasmic granules are usually demonstrable by the Nocht-Romanowsky method. Their nuclei are *vesicular*, rather coarsely reticulated, with one or two central nodal thickenings resembling nucleoli, but no true nuclei have as yet been demonstrated in these cells. The nuclei may be circular, or horseshoe-shaped (transitional leucocytes), or elongated. In Ehrlich's triacid solution the bodies of mononuclear basophile cells stain very faintly red. (Plate I.)

3. **Polynuclear*** **leucocytes** are two or three times as large as the red cell. Their protoplasm is reticulated and possesses as integral parts of the reticulum protoplasmic neutrophile granules in considerable number. The reticulum is otherwise very slightly basophilic, and may be demonstrated, with basophilic nodal thickenings or granules, by methylene blue. (Plates V. and VII.) Their nuclei are elongated and constricted, or composed of two or more lobes usually connected by threads of chromatin. These lobes are coarsely

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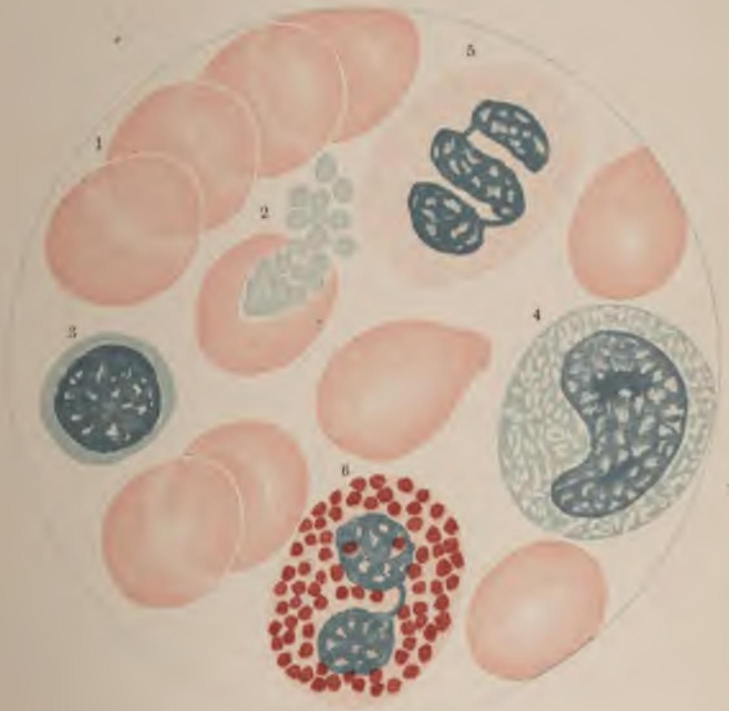
* The attempt to displace Ehrlich's old term "polynuclear" by the unfortunately derived "polymorphonuclear" seems to the writer ill-advised. The former designation has become firmly established, never leads to confusion, and seeing that polymorphism characterizes nuclei of other mononuclear cells, and that the lobes of the neutrophile cell are often separate, there appears to be no gain in accuracy by such a change.

PLATE II.



Normal Blood. (Eosin and Methylene Blue.)

Fig. 1. Normal rouleau.
Fig. 2. Red cell, extruding slightly basophilic (nuclear) contents as blood plates.
Fig. 3. Lymphocyte.
Fig. 4. Large mononuclear leucocyte with finely reticular cytoplasm.
Fig. 5. Polynuclear neutrophile leucocyte, granules unstained.
Fig. 6. Eosinophile leucocyte.

reticulated and usually possess a central nodal thickening, but true nucleoli have not been demonstrated. The lobes may become completely separated from each other.

Arnold,[2] Schur and Lowy, and Hirschfeld[2] have described the basophilic granules of the polynuclear leucocytes, the latter observer suggesting that they represent a basophile substratum of the neutrophile granules, and that their presence indicates that the cell is young. The writer regards it as uncertain that these granules have any connection with the neutrophile granules, since they exist in all polynuclear cells, and stages transitional to neutrophile granules apparently do not exist.

Sherrington[2] finds that in shed blood the nuclei of polynuclear leucocytes may reassume the spheroidal form, but it is to be doubted if this change ever occurs in the circulating blood.

4. **Eosinophile leucocytes** vary in size from that of lymphocytes to that of polynuclear leucocytes. Their protoplasm contains large strongly acidophile granules which are believed to be integral parts of a cytoreticulum (Heidenhain, Gulland). Their nuclei are coarsely reticulated and usually bilobed, the lobes are more often separate than in the neutrophile cells, and they stain rather faintly with nuclear dyes.

5. **Mast-cells** are mononuclear or polynuclear cells of different sizes, whose characteristic feature is the presence of large and small strongly basophile granules. These granules do not reflect the pure color of many stains, but are *metachromatic*, especially with thionin. A few mast-cells are always to be found in normal blood, but when present in any considerable numbers they are pathognomonic of myelogenous leukemia. (Plate XI.) Levaditi describes small acidophile granules in mast-cells. The mast-cell of the blood is entirely different in appearance from the mast-cell of the tissues.

Leucocytes in Pathological Blood. Besides the above forms of colorless cells which alone are present in normal blood, other types of cells are seen in the circulation in disease.

Myelocytes are mononuclear cells with neutrophile or with eosinophile granules. Three types of myelocytes should be distinguished:

(*a*) EHRLICH'S MYELOCYTE is a medium-sized cell with pale, usually central nucleus, and neutrophile granules. It is found in many morbid conditions, especially in leukemia and secondary anemia. (Plate VIII.) Ehrlich has described as "neutrophile pseudolymphocytes" very small myelocytes with densely staining nuclei, which were seen in hemorrhagic smallpox.

(*b*) CORNIL'S MYELOCYTE is a large cell, much larger than a polynuclear leucocyte, with pale eccentric nucleus and neutrophile granules. It is found almost exclusively in myelogenous leukemia, and less frequently in v. Jaksch's anemia. (Plate VIII.)

(*c*) EOSINOPHILE MYELOCYTES may resemble the eosinophile cells of normal blood, except that their nuclei are single. Such cells are abundant in myelogenous leukemia, occur not infrequently in v. Jaksch's anemia, and have been found in myxedema by Mendel, in some infectious diseases by Turck, and in pernicious malaria by Bignami. Or, their granules may be of excessive size, in which case they are pathognomonic of myelogenous leukemia. (Plate VIII.) Most myelocytes possess true acidophile nucleoli (Jolly).

The Classification of Leucocytes. Although the leucocytes were discovered by Nasse in 1835, the first scheme of classification based upon their supposed points of origin was offered by Virchow, who divided the colorless cells into *lymphocytes* derived from the lymph nodes, *splenocytes* from the spleen, while polynuclear cells he regarded as developmental forms of the mononuclear. Similarly, Einhorn, a pupil of Ehrlich, classed the leucocytes as: (1) lymphocytes, small and large, derived from the lymph nodes; (2) myelogenous cells (eosinophiles) from the marrow, and (3) large mononuclear, transitional, and polynuclear cells, from spleen or marrow.

In 1865 M. Schultze described the leucocytes as (1) non-granular (large and small mononuclears); (2) finely granular (neutrophiles), and (3) coarsely granular (eosinophiles and mast-cells). He believed that all granules and cells represent developmental forms of one series.

Lowit's classification, based on the morphology of the nucleus, included small and large mononuclear cells (lymphocytes), "transitional" leucocytes, and polynuclear leucocytes. Regarding solely the nucleus, Lowit found no difficulty in deriving all leucocytes in one series of cells. It remained for Ehrlich to establish the essential distinction between leucocytes by demonstrating specific microchemical reactions in the granules of Schultze.

Ehrlich divided the aniline dyes into three main groups: (1) Basic dyes, as hematoxylon, methylene blue, thionin, etc., act as bases, uniting, with selective power in the order named, with the acid principles of cells (nucleinic acid). (2) Acid dyes, as eosin, fuchsine, aurantia, act as acids, and unite with the basic principles of cells. (3) Neutral dyes. When certain basic and acid dyes are mixed a compound is formed of modified staining qualities which unites with certain cell structures not readily stained by other methods. Such a mixture Ehrlich calls a *neutrophile stain*, an example of which is the triacid mixture. Neutral red seems also to fall in this class.

According to their reactions to these dyes the granules of leucocytes in human blood may be divided into three main groups, basophile, acidophile (oxyphile), and neutrophile, and on these grounds the present classification of leucocytes is based, as follows:

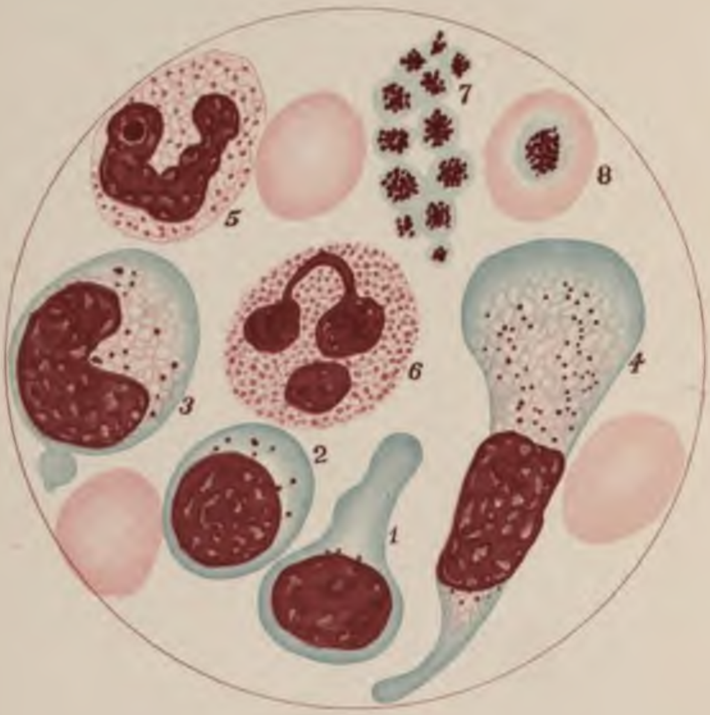
1. **Basophile Cells**: MAST-CELLS (*γ-granules*). Granules strongly basophile. LYMPHOCYTES. Protoplasm strongly basophile. LARGE MONONUCLEAR LEUCOCYTES. Protoplasm slightly basophile.

2. **Neutrophile Cells**: POLYNUCLEAR LEUCOCYTES. Neutrophile (ε) granules. MYELOCYTES. Neutrophile granules.

3. **Eosinophile Cells**: EOSINOPHILE LEUCOCYTES. Large acidophile granules (*α-granules*). EOSINOPHILE MYELOCYTES. Large acidophile granules.

Ehrlich also described β-granules which are amphophile, stain by both basic and acid dyes, and are found in some marrow cells, and δ-granules which are small basophile granules said to occur in some mononuclear cells.

PLATE III.



Normal Blood. (Fixed in Alcohol. Stained two hours by the Nocht-Romanowsky Method.)

Fig. 1. Ameboid lymphocyte. From a case of malaria, blood on warm stage under a cover-glass twenty minutes.

Fig. 2. Lymphocyte with large granules.

Fig. 3. Large lymphocyte with violet-stained cytoreticulum, large and small granules, and basophile cell border. Bud-like pseudopod.

Fig. 4. Ameboid large lymphocyte.

Fig. 5. Polynuclear leucocyte with cytoreticulum and scanty granules.

Fig. 6. Polynuclear leucocyte with numerous granules.

Fig. 7. Group of blood plates.

Fig. 8. Blood plates forming in red cell.

It is impossible here to consider all the objections raised against the specific quality of the reactions demonstrated by Ehrlich, and especially the limitations shown to exist in the application of his principles to other tissue cells. At present it must be admitted that while there are rather narrow limits between which the reactions of granules may vary, yet each class of granules is peculiar to one cell, and transitional stages have not been demonstrated, while the separation of leucocytes according to the chemical character of their protoplasm is a much more exact classification than one on any other basis. While the original views attributed to Ehrlich have suffered considerable modification since it became evident that the granules of many tissue cells, though of variable composition, may give identical reactions to dyes, and that the granules of the same leucocytes may stain differently, yet the wisdom of his classification is shown by the evidence which has accumulated regarding the significance of these granules.

The classification and nomenclature of Kanthack and Hardy is often employed in the discussion of leucocytes. These authors describe (1) lymphocytes; (2) hyaline cells, which are the large mononuclear leucocytes of Ehrlich, which cells, however, usually contain the granules described above; (3) finely granular oxyphile cells, which are the neutrophile and amphophile leucocytes of Ehrlich; (4) coarsely granular oxyphile cells, or eosinophile leucocytes; (5) coarsely and finely granular basophile cells, which are the mast-cells.

Finer Structure of Leucocytes. Recent studies have considerably increased the knowledge of the minute structure of leucocytes.

For many years opinions have been at variance regarding the structure of the protoplasm of basophilic cells. It is now evident that the bodies of these cells, while showing the reticular structure of ordinary protoplasm, with fine nodal thickenings, also contain a special variety of granules. The nodal thickenings do not, however, have the sharp character of the other granules of leucocytes. It has been shown by Arnold, Gulland, and others, that the neutrophile and eosinophile granules are connected by threads with the reticulum of their cells, and are thus integral parts of this reticulum (plasmatic).

Under suitable conditions, in thinly spread, rapidly dried, densely stained cells, it is apparent that the cytoreticulum of large mononuclear, neutrophile, and eosinophile cells is directly continuous with the nuclear network and that the clear space sometimes appearing to separate nucleus from body is artificial (Gulland).

Centrosomes have been demonstrated in the leucocytes of lower vertebrates by Flemming, Arnold, Van der Stricht, Hermann, Heidenhain, and in human blood cells by Gulland and Levaditi. These bodies show no special affinity for any dyes, but are best demonstrated by basic stains. They have been stained by safranin and gentian violet (Flemming) and by methylene blue (Gulland). In human blood they are extremely minute, and their demonstration appears to be a very uncertain undertaking.

Significance of Cell-granules. The theory of the higher significance of cell granules was first clearly stated by Altmann, although it was suggested by Ehrlich in his early studies of the granules of leucocytes. Instead of being degenerative or excretory products it is now apparent that *they are a secretory product, and represent the centre of the specific function of the cell.*

Strong evidence in favor of this view was furnished in the demonstrations by Heidenhain, Arnold, Gulland, and others, that the granules are *not paraplastic*—that is, loose particles separate from the cytoreticulum, like fat, melanin, etc., but are plasmatic—*i. e.*, integral parts of the cytoreticulum. The specific quality of the granules is also indicated by their chemical and tinctorial reactions. The differential chemical characters of the neutrophile and eosinophile granules will be considered later. (See Chapter V.) Although the granules of every cell of the same type may not all stain exactly alike, yet when submitted to fine chemical tests one cell is found to contain only one kind of granule. The studies of Knoll, Hirschfeld, Kurloff, Gruneberg, Rawitz, and Hesse, upon the leucocytes of lower animals have shown that the wandering cells of vertebrates and invertebrates possess granules which are peculiar to each animal, and are strictly limited to separate types of cells. Ehrlich regards the opposite behavior of different cells under different chemotactic influences as evidence of the specific nature and function of each variety of leucocyte and of its peculiar granule.

While the above considerations point to the secretory nature of the granules, the exact function of the secretion has not been demonstrated. Altmann believes that they furnish oxygen for the metabolic processes of the cell. Ehrlich once (1891) regarded them as a sort of reserve material destined for use within the cell, but has not expressed himself recently on this point. Hankin,[1] Kanthack, Hardy, and Keng, apply the term *alexines* to the secretory granules of leucocytes, and believe that they are destined to be thrown out into plasma or lymph and to exert a bactericidal or antitoxic influence. Sherrington and most observers cannot find sufficient ground for the acceptance of this view. Metchnikoff[1] refers to the granules as reserve material, but does not point out their exact destination.

Arnold[3] has gathered much interesting evidence regarding the origin and significance of cell granules, some of which speaks against their specific quality and constant character, and, therefore, against the plan of classification of Ehrlich. Arnold finds that fat and hemosiderin may be absorbed by leucocytes and assume the same plasmatic position as the cell granules. He claims that under some conditions the neutrophile or eosinophile granules may become transformed into fat or hemosiderin. In his studies of the marrow cells he emphasizes the changeable character of the granules of leucocytes and the very common occurrence of more than one kind of granule in the same cell.

Numbers of Leucocytes. The first estimates of the number of leucocytes in the cubic millimetre of blood were made by Samuel and Welcker and were generally accepted for some years. Malassez, with his improved method, considerably reduced these older figures. With the introduction of Thoma's instrument still more accurate estimates were secured which remain undisturbed at the present time, and agree fully with the more recent results obtained by Rieder, Reinert, and Limbeck.

The following table is compiled from the chief contributions on this point:

Observations on Normal Numbers of Leucocytes.

	Average.	*Maximum.*	*Minimum.*
Samuel	14,000		
Welcker	13,432	14,925	12,857
Moleschott	......	14,000	12,605
Malassez[2]	......	7,692	3,750
Duperie	4,545		
Hayem	6,000		
Bouchout, Dubrisay	6,116		
Grancher	......	9,000	3,000
Hirt			
Thoma, Lyon	8,388	10,590	6,784
	5,464	7,066	4,430
Halla	7,583	10,106	4,960
Tumas	6,200	9,600	4,800
Sorensen	......	10	
Patrigeon	......	10,000	2,000
Reinecke	7,351		
v. Jaksch	7,482		
Limbeck	8-9,000		
Rieder, adults	7,680	9,600	4,200
Rieder, children	9,960	12,400	7,200
Reinert, 6 A.M.	5,125		
Reinert, 4 P.M.	8,262		

In estimating the leucocytes in both health and disease one is confronted by a great variety of disturbing factors, including the causes of distinct physiological leucocytosis, the individual peculiarities of the subject, and the ordinary variations in the local condition of the part from which the blood is taken. All that has been said regarding accidental variations in red cells applies equally to leucocytes, and one must carefully consider the effects of vasomotor phenomena, of changes in the volume of plasma, and of the presence of inflammation or edema. It should be remembered that while the leucocytes remain nearly uniform in the great vessels their proportions in the capillary circulation may change more rapidly than those of the red cells, owing probably to chemotactic influences. The most common sources of error may be avoided by taking specimens about four hours after a meal and at the same hour each day.

Proportions of Various Forms of Leucocytes. The proportions of the different forms of leucocytes in normal blood are even less fixed than their numbers. Ehrlich's figures may well serve as a standard for healthy adults.

Lymphocytes, 22 to 25 per cent.

Large mononuclear and transitional leucocytes, 2 to 4 per cent.

Polynuclear neutrophile leucocytes, 70 to 72 per cent.

Eosinophile cells, 2 to 4 per cent.

Mast-cells, 0.5 to 2 per cent.

The chief variations from these limits which deserve mention are the maximum percentages given by Rieder for lymphocytes (30 per cent.) and by Limbeck for polynuclear leucocytes (80 per cent.). In childhood the proportion of lymphocytes is usually much increased (55 to 66 per cent.), and that of polynuclear cells correspondingly diminished (28 to 40 per cent.). (Gundobin, Rieder.)

Degenerative Changes in Leucocytes.

While degenerative changes in leucocytes have always been recognized as necessary processes in degenerating tissue in which the leucocytes were lodged, references to acute degenerative changes in leucocytes of the circulating blood have been so scanty as to lead some writers to deny their existence. It, therefore, becomes necessary to consider briefly the general evidence at hand concerning the occurrence and significance of such changes in circulating leucocytes.

It was perhaps Lowit's claim that leucocytolysis necessarily precedes leucocytosis, which called special attention in recent years to the destruction of leucocytes in the circulation. Although Lowit's claim cannot be fully supported, the fact that the majority of leucocytoses are preceded or accompanied by the destruction of many leucocytes in the circulation has been amply proven by the work of Uskoff, Botkin, Gabritschewsky,[1] Engel, Klein, Goldscheider and Jacob, and many others.

E. Botkin followed up the subject in a series of experimental and clinical studies and showed that in 1 per cent. solution of peptone and in the fresh plasma of various infectious diseases, all leucocytes dissolve and disappear with varying rapidity. The lymphocytes proved most resistant. The changes described by Botkin were of an extensive variety, but the principal features included the formation of coarse granules in mononuclear cells; extrusion and loss of granules from polynuclear cells; formation of vacuoles and clefts; fragmentation and solution of cell bodies; formation of blood plates; swelling, fading, shrinkage, subdivision and final disappearance of nuclei. Comparing these experimental changes with those observed by himself or reported by others in infectious diseases and in leukemia, Botkin declared that *no examination of the blood can be considered complete which leaves out of account the degenerative changes in leucocytes.*

The principal contributions which seemed then (1896) to justify Botkin's claim were those of Hankin, who found in the extrusion and loss of granules of the rabbit's leucocytes the source of bactericidal alexines; of Haetaguroff, who observed various degenerative changes in the leucocytes of typhoid fever; of Engel, who described a variety of changes in the leucocytes of an anemic child, and of Gumprecht, who called special attention to the pathological changes in the leucocytes of leukemia.

In 1895 the writer described degenerative changes in the leucocytes of diphtheria. Similar lesions have since been reported by File, who regarded many of the altered cells as dead, and by many others.

In the absence of more extended observation it seems best to merely describe rather than attempt to accurately classify the pathological changes in leucocytes, yet a tentative classification may be employed for convenience sake.

Acute Degeneration of Leucocytes. This process is best demonstrated in acute infectious diseases, especially in severe cases.

As seen in diphtheria the alterations affect both body and nucleus.

In diphtheria the writer believed he could detect an *increased acidophile staining tendency* in the neutrophile granules, a conclusion which he is disposed to retain, seeing that Kanthack had previously found that the pseudoeosinophile granules of the rabbit may be made to show increased acidophile tendency and higher refractive quality by the injection of minimum immunizing doses of microbic poisons. This observation, however, has not been further confirmed. That this change when it occurs belongs in the degenerative series is not clear, but it may be seen in cells which show other distinct signs of degeneration.

Diminution in the number of neutrophile granules is commonly observed in the polynuclear cells in acute leucocytosis. This abnormality may progress until very few granules are left, but their complete absence is seen principally in chronic leukemia. It is usually associated with marked nuclear changes. Eosinophile cells may be similarly altered. Arneth has described cases of pernicious anemia and leukemia in which very few of the polynuclear leucocytes or myelocytes showed granules.

Swelling and fragmentation of the bodies of leucocytes is commonly observed in acute leucocytoses. It is probable that many of the torn and distorted cells seen in dry preparations have undergone degenerative changes which have favored their complete destruction in smearing. These cells have been described as "leucocyte shadows" (Klein), a term well suited to recall their torn bodies, scattered granules, and faded nuclei.

The reticulum of large and small mononuclear cells in leukemia and in severe acute toxemia may grow *coarser* and at times *granular*. Botkin has described the appearance of granules in the dissolving lymphocytes of shed blood. Some of the lymphocytes of leukemia appear to be distinctly granular. Ehrlich depicts the separation of small peripheral fragments of the protoplasm of lymphocytes. Globular projections from such cells are frequently encountered, but it does not appear certain that they represent degenerative plasmoschisis.

Nuclear changes appear to be the most significant of the lesions of acute degeneration of leucocytes. In fresh or dried specimens the nuclei stain less densely with basic dyes, their outlines are irregular and the lobes shrunken. The degeneration may follow the type of karyolysis, with swelling and loss of chromatin, or of karyorrhexis with hyperchromatosis and subdivision of lobes (Gumprecht). In acute leucocytosis the former type is more usual, but in leukemia the latter form is abundantly seen. (See Plate X.) While the lobes of the normal polynuclear leucocytes are almost invariably connected with a thread of chromatin, many of the cells in severe acute leucocytosis show complete subdivision into three to six separate segments.

Chronic Degeneration of Leucocytes. In diseases like leukemia in which peculiar chronic changes in white cells are prominent, many of the alterations commonly seen in acute degeneration are also present. Thus, in myelogenous leukemia one finds leucocytes showing loss of granules, presence of vacuoles, granules of glycogen, faded irregular nuclei, and distortion and fragmentation of cell bodies. Some of the cells in leukemic blood, however, exhibit changes which at least in their abundance are characteristic of the disease and which seem certainly to be the result of chronic processes.

Complete loss of neutrophile granules may be observed in many cells, both polynuclear and mononuclear, in leukemia, less frequently in other conditions. The cell body remains slightly acidophile, while the nucleus commonly shows degenerative changes. (Plate X.)

Complete subdivision of nuclei of polynuclear cells into six to ten hyperchromatic segments is a rather characteristic appearance in leukemic blood, and is usually associated with complete loss of granules. (Plate X.)

Hydropic Degeneration. In fresh blood many leucocytes may be found whose nuclei are indistinct or invisible and whose bodies contain many large and small vacuoles bounded by homogeneous or granular reticulum. These disintegrating cells are specially abundant in malaria and leukemia. In malarial blood when such cells are pigmented, the prominent vacuoles, opaque granules, and streaming movements common in dying protoplasm strongly suggest the appearance of vacuolated parasites. In stained specimens they are found to be composed of pale staining remnants of nuclei. (See Plate IX., Fig. 3.)

Hydropic degeneration of nuclei is practically limited to some peculiar leucocytes, both mononuclear and polynuclear, of leukemia. (Plate X.)

Fatty Degeneration. Fatty degeneration of leucocytes has often been referred to as a part of the changes in pathological blood. The writer has seen globules in leucocytes blackening with osmic acid in sections of blood in the vessels of fatty viscera, very rarely in circulating blood. The presence of these globules in leucocytes is not a certain indication that they have resulted from the altered albuminous constituents of the cell, nor does it necessarily indicate a degenerative process, so that in the absence of further evidence the writer is unable to include this form of degeneration among those affecting white blood cells. Even when fat is abundant in the plasma the leucocytes are commonly free from it (Gumprecht).

Arnold[3] describes in the leucocytes of the frog plasmatic and paraplastic granules of fat which were absorbed or englobed from the circulating medium.

Glycogenic degeneration has been considered in Chapter II.

Perinuclear Basophilia. In 1894 Neusser (and later Kolisch) described the appearance of basic staining granules about the nuclei of polynuclear and other leucocytes, which he termed as above and claimed to be a somewhat pathognomonic sign of the uric acid diathesis. At the time this observation was announced Lowit stated that perinuclear basophilia was an artifact which he had kept in mind for some ten years. Nevertheless, Neusser's perinuclear basophilia brought considerable notoriety to the Vienna school during several years. Its lack of clinical or pathological significance has been fully demonstrated, among others, by Futcher and by Simon.

Blood Dust. Hemokonia.—The older histologists have rather uniformly described in normal plasma minute spheroidal or spindle-shaped bodies of undetermined origin (Bizzozero, Landois, Hayem). Recently, Müller[2] has drawn special attention to their occurrence, describing them as spheroidal, or dumb-bell, or spindle-shaped granules $\frac{1}{4}\mu$ to 1μ in diameter, and exhibiting active Brownian movements, but no locomotion. They are not fat, being insoluble in alcohol and ether and not stainable with osmic acid, and they appear to have no connection with the process of fibrin-formation. Their identity with the granules of leucocytes was considered by Müller, and is claimed by Stokes and Wegefarth, who found them to increase in number as the specimen stood, and observed that in different animals their size varied with that of the granules of the leucocytes. Nicholls identifies them with leucocyte granules on microchemical reactions. Müller found them unusually abundant in a case of Addison's disease, and scanty in starvation and cachexia, but no clinical significance has been demonstrated for them. If not derived from leucocytes, they may be in part the extruded contents of fragmenting red cells or precipitated albuminous particles from the plasma.

LEUCOCYTOSIS.

Nomenclature. An increase of leucocytes distinctly above normal limits is called *leucocytosis.* When the increase affects principally the polynuclear leucocytes, the condition is termed *polynuclear leucocytosis* or simply *leucocytosis.* An increase of lymphocytes alone is called *lymphocytosis.* Sometimes several varieties of cells are found in increased numbers, when the term *mixed leucocytosis* is used. *Eosinophilia* is perhaps an admissible word to designate an increase of eosinophile cells.

A distinct diminution of leucocytes is either so phrased or termed *hypoleucocytosis* in contrast to *hyperleucocytosis,* which signifies an excess of cells.

Classification of Leucocytoses. Rieder first employed the following natural divisions of the subject :

I. **Physiological Leucocytoses.**
  Leucocytosis of digestion.
  Leucocytosis of pregnancy.
  Leucocytosis of the newborn.
II. **Pathological Leucocytoses.**
  Post-hemorrhagic leucocytosis.
  Cachectic leucocytosis.
  Antemortem leucocytosis.
  Inflammatory leucocytosis.

Each variety of leucocytosis has a somewhat particular significance. That of the inflammatory type will first be considered.

Significance of Inflammatory Leucocytosis. It was early seen that the most typical occurrence of transient leucocytosis is limited to certain acute infectious diseases, and here its significance has been the subject of much study and discussion, the extent of which has shown this subject to be one of the broadest of biological problems.

A very simple illustration of a principle believed to exert prominent influence in the behavior of leucocytes is found in the actions of certain myxomycetes in the presence of chemicals. Stahl found that *Ethalium septicum* placed on a moistened surface close to a drop of infusion of oak bark moved actively toward and into the infusion, but moved actively away from a solution (5 per cent.) of glucose. He also noted that the *plasmodium of Fuligo*, which at first moves away from a 2 per cent. solution of salt, will later, if needing water, advance and enter the solution. Pfeiffer called this varying sensitiveness of protozoa toward chemicals *chemotaxis*, and the above observations may serve to illustrate *positive* and *negative* chemotaxis and the transformation of a negative into a positive chemotaxis. Pfeiffer also extended these observations to bacteria, finding that nearly all nutritive substances exert a positive attraction for bacteria, which is determined not by movements of diffusion of the liquids, but by the specific nature of the chemical substances.

A chemotactic influence of bacteria upon leucocytes apart from the phagocytic tendencies of Metchnikoff was pointed out by Peckelharing, among the first, who inserted beneath the skin of frogs pieces of parchment containing *anthrax*, and found them after some hours surrounded by leucocytes. Gabritschewsky[2] showed that the attractive agent was dissolved in the fluids of these cultures. An extensive mass of observations has accumulated, showing that there is a wide variety of bacteria and bacterial and chemical products which exert a specific positive attraction upon leucocytes. In some instances a preliminary repulsion is succeeded by a positive chemotaxis, but the examples of distinct negative chemotaxis of bacteria upon leucocytes are so uncertain as to lead Kanthack to conclude that probably no bacteria actually repel leucocytes, though some very rapidly destroy the attracted cells, so that the surrounding area is comparatively free from them. An apparently clear example of negative chemotaxis is found in the action of the bacillus of chicken cholera in some susceptible animals, while there are a great many examples of failure of positive chemotaxis (*Bacillus tuberculosis, typhosus.*)

A distinctly new element was added to the knowledge of chemotaxis when it was first clearly shown, by Leber,[1] that chemotactic action of bacteria is exerted upon leucocytes *at a distance*. On placing within the anterior chamber of the eye of the rabbit a fine tube filled with a substance extracted from cultures of *Staphylococcus aureus*, Leber found that the leucocytes would promptly overcome the action of gravity and force a way for a considerable distance through lymph channels in order to enter the tube.

The subject was again considerably enlarged in the experimental production of leucocytosis by Limbeck, who pointed out that along with the local afflux of leucocytes following subcutaneous injections of bacterial cultures, there is a marked increase in the general circulation, which in fact precedes the local changes.

This action upon distant blood-producing organs causing an outpour of leucocytes has been variously interpreted. It has been referred by most authors to the direct chemotactic action of the virus distributed through the circulation. Limbeck regarded the intravascular leucocytosis as somewhat in the nature of a forerunner and integral part of the purulent exudate which gathers at the point of inoculation. Weiss carries this idea still further, actively maintaining that leucocytes in inflammatory conditions are formed in various tissues whence they pass into the blood. Leucocytosis is, therefore, not of uniform significance and origin, but is the expression of various underlying tissue changes in disease. Lowit believed that the increase of leucocytes merely represents a *regenerative effort on the part of the marrow* to replace destroyed leucocytes, his observations supporting Virchow's original view of the origin of leucocytosis. Buchner and Romer also refer leucocytosis solely to the *increased production of the cells under the stimulus of bacterial proteins.* That an *increased flow of lymph* occurs in many cases of inflammatory leucocytosis, especially in that in which the lymphocytes are increased, is indicated by the demonstration of a special lymphogogic property in bacterial proteins (Buchner, Gärtner, and Romer).

Schultze, Rieder, and Goldscheider and Jacob, have held more or less strictly

to the opinion that the leucocytes are not greatly multiplied during leucocytosis, but, on the contrary, are merely overabundant in peripheral vessels, or to a *moderate extent* are drawn into the circulation from the depots of ready-formed cells in the marrow.

It is impossible here to consider in detail the basis of these various hypotheses. Of the last mentioned it must be said that there is abundant evidence both of a new production of leucocytes in the viscera and of an increased outpour of these cells during leucocytosis.

Although the evidences of new formation of leucocytes in the circulating blood, lymph nodes, and marrow, found by Schmidt, Lowit, and Rieder, seem to them inadequate to account for a great increase in the number of these cells, this result may be referred to the uncertainty regarding the origin of leucocytes, to the mistaken opinion that polynuclear leucocytes are derived from lymphocytes, and to the difficulty of demonstrating early proliferative changes in such widely distributed fields as the blood and marrow. Yet Lowit[2] could find considerably increased numbers of mitotic and amitotic nuclei in circulating leucocytes during leucocytosis.

Recent studies of the marrow during experimental leucocytoses have fully established the existence of greatly increased cellular proliferation beginning in the early stages of leucocytosis, and, in prolonged cases, completely transforming the histological appearance of this tissue. The writer in 1895 compared the marrow of typhoid fever with that of pneumonia and other exudative diseases in a series of cases, and found a very striking difference in the pulp cords in the two conditions, leucocytosis being accompanied by marked cellular hyperplasia, affecting principally the neutrophile myelocytes.

Rogers and Josue found, forty-eight hours after the injection of *staphylococcus aureus* into rabbits, marked congestion and cellular hyperplasia of the marrow cords affecting principally the nucleated red cells and eosinophiles, but by the third day these cells disappeared, and neutrophile myelocytes with many giant cells formed the bulk of the now greatly hypertrophied cords. These observations have been verified and extended by Muir and many others.

The sole ground that remains to those who deny that inflammatory leucocytosis is essentially a new formation of leucocytes is the probability that many leucocytes drawn into the circulation *during the first hours* of leucocytosis are ready-formed in the marrow.

Of the other hypotheses it still appears that each contributes a portion of the truth. Probably the combined influences of chemotaxis, and of the new formation of leucocytes stimulated by bacterial proteins, etc., are chiefly responsible for the general afflux of white cells. The destruction of leucocytes has repeatedly been shown to be a subordinate factor and entirely too slight to stimulate the production of new cells through ordinary channels, as suggested by Lowit.

Limbeck's early belief in a close connection between local exudate and general leucocytosis is at least partially true, and serves to emphasize, as Grawitz points out, that many tissues, to an extent, and not merely the blood-forming organs, furnish leucocytes to the circulation.

In so far as chemotactic influences are concerned in leucocytosis it should be remembered that the essential nature of the attractive force remains entirely unknown.

In studies upon the visceral changes in leucocytosis the writer became convinced that some phenomena now attributed to an obscure chemotactic influence may be fully explained on purely mechanical principles. This statement applies especially to the sifting of cohesive leucocytes by swollen capillary endothelium, and to a less extent to the outpour of new-formed leucocytes from the marrow, but in the phenomena of local exudates other factors seem undoubtedly to be concerned.

The Course of Leucocytosis. The experimental study of leucocytosis has greatly increased the knowledge of the order and significance of the phenomena connected therewith. It was first observed by Werigo that the inoculation of animals with bacterial cultures is followed within five or ten minutes by a pronounced diminution of circulating leucocytes (*hypoleucocytosis*), by the deposit of the bacteria in the capillaries of the lungs, liver, and other viscera, and by their complete disappearance from the blood.

Approaching the subject from another standpoint, Goldscheider and Jacob, and the writer, found that along with the deposit of bacteria the leucocytes are also sifted out of the circulation and permanently lodged in visceral capillaries, principally in the lungs and liver. The writer also partially agreed with Lowit that actual destruction of leucocytes follows such intravascular inoculations, especially with large doses, but did not find, contrary to Lowit, that leucocytolysis in disease plays more than a very subordinate part in ridding the blood of white cells. The initial hypoleucocytosis has been observed very constantly to precede the appearance of leucocytes in increased numbers, and the same phenomenon has been observed in the course of many natural infections in the human subject.

In experimental studies it has been found that many animals perish during the stage of hypoleucocytosis, especially when an active virus is introduced into a susceptible animal. Thus the inoculation of guinea-pigs with virulent cultures of diphtheria is followed by death within twenty-four to forty-eight hours, *during persistent hypoleucocytosis*, while in the less susceptible rabbit the same treatment usually kills the animal, but not before leucocytosis has become well established. *In general, the increase of leucocytes follows the initial diminution more rapidly in those animals in which the course of the infection is more favorable.* The same rule is found to hold in the human subject, initial hypoleucocytosis, sometimes persistent, having been observed in malignant cases of pneumonia (Kikodse and others), diphtheria (Billings), septicemia, etc., and its unfavorable import in these diseases has been fully demonstrated.

In infections of ordinary virulence hypoleucocytosis is established within one-half to two hours after the initial diminution, and Goldscheider and Jacob succeeded in exciting pronounced leucocytosis without any demonstrable initial decrease in cells. During the course of febrile reaction to exudative processes, and sometimes during a considerable period thereafter, hyperleucocytosis persists. *The grade of leucocytosis has been found to vary often with the height of the temperature, more closely with the extent of the exudate, but as might be expected from its recognized significance, measures more exactly the reaction of the system to the infectious agent*

Throughout the stages of inflammatory leucocytosis it is the polynuclear leucocytes which are chiefly or exclusively concerned with the process. During hypoleucocytosis these cells, and as the writer finds, the large mononuclear cells, are greatly reduced, having been caught in the capillaries, while the lymphocytes remain in large proportion. Very soon, with the increase in total numbers, the polynuclear cells are found in excessive proportions (80 to 95 per cent.), and even when the total increase is not great the excess of polynuclear cells is so constant as to furnish at times significant information regarding the character of the infection.

The hyperemia and excitation of the marrow is indicated by the presence of normoblasts (Timofjewsky) and of a few myelocytes which have now been found in many severe leucocytoses, especially in diphtheria (Engel).

An absolute increase of lymphocytes, sometimes of extreme grade, as observed by the writer in diphtheria, is often noted in the course of inflammatory leucocytosis, and is to be referred to special involvement of lymphoid tissues.

Ehrlich[3] holds that while polynuclear leucocytosis is referable to chemotactic influences acting from a distance, lymphocytosis is always the result of local irritation acting mechanically. The recent observations on the ameboid properties of lymphocytes and large mononuclear leucocytes indicate, however, that these cells may actively respond to certain chemotactic influences and emigrate from the vessels, and they have been described as passing through vessel walls in nephritis, through squamous epithelium in tonsillitis, and gathering in large numbers in the early stages of abscess formation (Janowsky). On the other hand, Marchand's conclusions, that these round cells in inflammation are chiefly derivatives of fixed tissue cells or of extravascular lymphocytes, represent the old and conservative opinion on this subject.

Relative or absolute lymphocytosis is seen in digestion leucocytosis (Rieder), in typhoid fever, with tumors of bone, and, in general, is a frequent feature of inflammatory leucocytosis in children.

During the subsidence of acute leucocytosis the neutrophile are sometimes replaced by a considerable proportion of eosinophile cells, as in pneumonia, septic processes, etc. The cause of this phenomenon is undetermined, but it has been shown to be of favorable import.

The behavior of the different cells in leucocytosis is largely in accord with their known characteristics elsewhere. The actively ameboid and phagocytic cells are chiefly affected, while the lymphocytes are much less frequently disturbed. An exception to this rule is found in the actively ameboid eosinophile cells, but these are

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The hyperemia and excitation of the marrow is indicated by the presence of normoblasts (Timofjewsky) and of a few myelocytes which have now been found in many severe leucocytoses, especially in diphtheria (Engel).

An absolute increase of lymphocytes, sometimes of extreme grade, as observed by the writer in diphtheria, is often noted in the course of inflammatory leucocytosis, and is to be referred to special involvement of lymphoid tissues.

Ehrlich[3] holds that while polynuclear leucocytosis is referable to chemotactic influences acting from a distance, lymphocytosis is always the result of local irritation acting mechanically. The recent observations on the ameboid properties of lymphocytes and large mononuclear leucocytes indicate, however, that these cells may actively respond to certain chemotactic influences and emigrate from the vessels, and they have been described as passing through vessel walls in nephritis, through squamous epithelium in tonsillitis, and gathering in large numbers in the early stages of abscess formation (Janowsky). On the other hand, Marchand's conclusions, that these round cells in inflammation are chiefly derivatives of fixed tissue cells or of extravascular lymphocytes, represent the old and conservative opinion on this subject.

Relative or absolute lymphocytosis is seen in digestion leucocytosis (Rieder), in typhoid fever, with tumors of bone, and, in general, is a frequent feature of inflammatory leucocytosis in children.

During the subsidence of acute leucocytosis the neutrophile are sometimes replaced by a considerable proportion of eosinophile cells, as in pneumonia, septic processes, etc. The cause of this phenomenon is undetermined, but it has been shown to be of favorable import.

The behavior of the different cells in leucocytosis is largely in accord with their known characteristics elsewhere. The actively ameboid and phagocytic cells are chiefly affected, while the lymphocytes are much less frequently disturbed. An exception to this rule is found in the actively ameboid eosinophile cells, but these are rarely found to show phagocytic qualities. The bearing of these facts upon the significance of leucocytosis will be considered later.

Relation of Leucocytosis and Phagocytosis to Immunity. The studies of Metchnikoff upon phagocytosis in lower animals have not only established the great importance of this process as a protective measure in the protozoa, but have gone far to explain the significance of leucocytosis in the higher metazoa. There is no room to doubt that *monads*, for example, englobe, digest, and destroy living threads of *leptothrix*, and the steps through which Metchnikoff has traced the process seem to show that in animals as high as frogs the leucocytes alone are responsible for the englobement and destruction of anthrax bacilli. In the higher vertebrates, however, other factors may very well supervene, and before accepting the phagocytic doctrine of leucocytosis in the human subject it is necessary to consider in some detail the extent and limitations of the process in higher vertebrates.

The process of phagocytosis, as described by Metchnikoff, begins with the chemotactic attraction of leucocytes toward bacteria, the englobement of the germs, often in the living virulent condition, and their digestion in stages which may be followed by changes in form and staining quality. The power to approach and englobe bacteria is obviously found in the ameboid activity of leucocytes. The power to digest bacteria is indicated by the morphological and chemical changes above mentioned, which indicate the breaking up of a bacillus into granules and its gradual loss of staining capacity. Moreover, a digestive ferment has been demonstrated in leucocytes by Rossbach and Leber,[1] and many investigators, especially Buchner,[2] have demonstrated very high bactericidal power in exudates rich in pus. That leucocytes actually exert these bactericidal powers in infectious diseases is strongly indicated by the different behavior of these cells in refractory and susceptible animals. It has been repeatedly shown that the leucocytes of susceptible animals which do not naturally attack certain bacteria may be made to do so by the immunization of the animal, as in the infection of mice and guinea-pigs with anthrax, and pigeons and rabbits with chicken cholera. That the englobed bacteria are alive and active has been shown by their movements within the surrounding vacuoles of the leucocytes, and by the subsequent development of the englobed bacteria when the leucocytes are placed in bouillon. (*Anthrax* in pigeons, *Vib. Metch.* in immune guinea-pigs.) Indeed, an *increase in virulence* has been found in cultures obtained from some bacteria passed through the leucocytes of refractory animals. Moreover, Lubarsch has shown that frogs' leucocytes are more actively attracted by living bacteria than by the same germ when dead.

It should be noted that drugs which inhibit the activities of leucocytes have been found to greatly accelerate the spread of certain infections. (*Opium in cholera*, Cantacuzene.)

The *histological study* of the reactive process at the seat of inoculation of a virus under various conditions has, also, furnished very strong support of the belief in the essential importance of phagocytosis. It has repeatedly been shown that in susceptible animals injections of highly virulent bacteria are, in general, followed by serous and bloody exudates which fail to limit the infection which becomes general and kills the animal, while the same treatment of immune animals is followed by highly purulent exudates, the limitation of the infection, and recovery (Gabritschewsky[3]).

Finally, the results of minute study of the behavior of various phagocytes should be considered. The two actively phagocytic leucocytes, the polynuclear neutrophile and large hyaline cells, do not attack all bacteria in the same manner. The former are less selective, englobing nearly all forms of bacteria. Yet the bacillus of leprosy is found almost exclusively within the large hyaline cells which do not attack streptococci or gonococci. Kanthack and Hardy have made important observations in this field. Examining a hanging drop of frog's lymph inoculated with a few anthrax bacilli, they found that the eosinophile cells were first attracted to the bacilli, that they discharged their granules upon coming into contact with the germs, and that degenerative changes soon appeared in the bacteria. Later the large hyaline cells surrounded the mass, the oxyphiles dropped away, and the altered bacteria were englobed and digested by the hyaline leucocytes. These observers found the same division of labor among leucocytes in various animals up to man, the hyaline cells acting as phagocytes of the celomic and lymphatic systems, and the finely granular oxyphile cells as phagocytes in the hemal system. They ascribe important bactericidal functions to the granules of eosinophile leucocytes in preparing germs for englobement by the ameboid phagocytes. Hankin also came to identical conclusions regarding the importance of eosinophile cells in phagocytosis in the rabbit.

Based upon these and many other similar observations, verified by many but actively combated by others, *Metchnikoff concluded that phagocytosis is the essential feature of inflammation, and the chief mechanism in immunity, and gave exclusively to the leucocytes and the endothelial and other phagocytic tissue cells a function of vast importance.*

It has been necessary, however, to modify the original claims of Metchnikoff in an important particular, that *an extracellular influence is exerted upon bacteria which is sometimes a necessary preliminary to phagocytosis.*

The steps by which this fact has been demonstrated may be briefly indicated.

Traube, Fodor, and others recognized that the destruction of bacteria in the living blood is often extremely active, and it was suggested that the fluid plasma, and not the leucocytes and endothelia, is the chief agent in freeing the animal organism from infectious germs. Grohman then demonstrated the bactericidal action of shed blood. Nuttall next found that during the process of inflammation, about the seat of inoculation in rabbits infected with attenuated anthrax, many bacteria which are not englobed nevertheless show degenerative changes, and he also found that blood serum and lymph possess very active bactericidal powers. These observations were verified and greatly extended by Nissen, Hankin, Behring, Buchner, Prudden, and Vaughan, who have fully established the presence both of bactericidal and of antitoxic principles.

It soon became apparent, however, that there exists a very close connection between these "defensive proteids" of blood and exudates, and the leucocytes. Hankin, Buchner, Kossel, and Vaughan have shown that a nucleo-proteid derived probably from the nuclei of leucocytes is the bactericidal agent in serum, exudates, and lymph. This bactericidal proteid belongs to the class of *enzymes*, is extremely unstable, is destroyed by heating to 55° to 65° C., and has been called by Buchner "alexine," and by Ehrlich "complement."

According to Hankin, blood serum ordinarily contains little of this agent, but after the second day of leucocytosis it is secreted by the eosinophile cells of rabbits' blood or is discharged from these cells during defibrination. Hahn and Van de Velde have shown rather conclusively that the alexines are a secretory product of the living leucocyte, and are not produced by the destruction of these cells, although they may be set free thereby. Hankin, Buchner,[3] Hahn, Bordet, Schattenfroh, Bail, and others have established the essential relation between the bactericidal power of exudates and their content in leucocytes, and have placed this relation beyond doubt by such demonstrations as that of Denys and Havel, that the blood and exudates of dogs lose their bactericidal power when freed by filtration or the centrifuge from intact leucocytes, but regain this power when the leucocytes are replaced.

The controversy regarding the so-called "*phenomenon of Pfeiffer*" has also ended in a way to favor the importance of leucocytes in the defence of the organism. Pfeiffer found that when cholera cultures are injected into the peritoneum of highly immunized guinea-pigs, the germs are broken up into granules, and to some extent dissolved by the peritoneal fluid before leucocytosis and phagocytosis have become fully established, but not, as some citations of this work would lead one to suppose, before the leucocytes present are actively engaged in phagocytosis.

Metchnikoff[2] promptly repeated this experiment, finding that the cholera spirillum flourishes in a hanging drop of the same peritoneal fluid which in the body is bactericidal, that the peritoneal fluid is really very rich in leucocytes; that if a great afflux of leucocytes is previously excited by injection of bouillon the phenomenon of Pfeiffer is suppressed, but the germs are englobed and destroyed just as quickly by the leucocytes; and he found, in general, that the phenomenon of Pfeiffer occurs only in animals whose leucocytes possess very active bactericidal and phagocytic qualities, and only in those situations where leucocytes are or have been abundant.

The discovery of the "phenomenon of Pfeiffer" placed beyond question that bacteria may be destroyed by entirely extracellular processes, a fact now not infrequently illustrated in the dissolution of bacteria in Widal's test. While Metchnikoff accepted this fact and altered his views as to the invariable necessity of phagocytosis, the application of his doctrine was extended rather than narrowed; for it still remains true that the bactericidal principles are elaborated principally in the leucocytes.

Since the development of Ehrlich's theories of immunity Metchnikoff and his school have succeeded in reconciling to a very large extent their somewhat opposing views regarding the mechanism of immunity.

In the destruction of bacteria Metchnikoff recognizes the interaction of two principles, *immune body* (*fixator*) and *complement* (*alexine*), but while admitting that the fixator circulates free in the plasma he claims that the alexine is liberated only on the destruction of leucocytes, and does not exist free in the plasma, while both are largely or exclusively the product of leucocytes. These conclusions have been reached by combining the morphological study of cells and tissues with the purely chemical observations of Ehrlich.

Observations Indicating that the Alexine is Not Free in the Plasma. Several observers have found that red cells placed beneath the skin of animals immunized against these cells are not dissolved until leucocytes begin to gather about them. In guinea-pigs possessing spermatoxin the spermatozoa are not dissolved in the animals' testicles, although in the test tube these same spermatozoa are dissolved by the serum of the same animal. In immunized animals hemolysis in the peritoneum may be prevented by washing the peritoneum with salt solution, thereby removing the alexine. Levaditi found that hemolytic serum is inert when obtained by centrifuging blood in paraffined tubes which prevents destruction of leucocytes, while these same red cells, loaded with fixator, may afterward be dissolved by the addition of normal serum obtained in the ordinary way, and containing alexine from disintegrated leucocytes. Walker also has found that serum drawn immediately after coagulation of the blood is very deficient in hemolytic properties as compared with that drawn after six to eight hours. Besredka finds that anticomplement serum acts upon the leucocytes preventing phagocytosis.

The chief action of the fixator (immune body) Metchnikoff believes to lie in stimulating phagocytosis, which is also facilitated by agglutination of bacteria or foreign cells. The chief source of the fixator he believes to be in a secretion from the leucocytes, and not from the specific cells affected by the immunization, since he obtained an active spermatoxic serum from a castrated animal.

Macrocytase, Microcytase. Metchnikoff brings forward strong evidence to show that the large lymphocytes (*macrophages*) possess a different function in immunity from the polynuclear leucocytes (*microphages*).

Exudates rich in polynuclear leucocytes (microcytase) are actively bactericidal, but not hemolytic, while exudates containing many macrophages (macrocytase) are actively hemolytic, but not bactericidal. Injections of red cells into the pleural cavity cause exudates rich in macrophages, while injections of bacteria excite exudates rich in polynuclear cells, each of the exudates proving antagonistic only to the agents injected.

On injecting cholera bacilli into the peritoneal cavity of an immune animal it was found that both macrophages and microphages englobed the bacteria, and while those within the microphages perished, those in the macrophages multiplied and destroyed these cells. From such observations Metchnikoff concludes that the macrophages act only on foreign body cells, and the microphages only on bacteria.

That the macrophages are the chief producers of antitoxins he deduces from the fact, among others, that the alligator, whose leucocytes are almost exclusively macrophages, is a very active producer of antitoxins.

The foregoing review of the steps through which the present conception of the relation of phagocytosis to immunity has been reached, while defective in important and highly interesting details has been rendered necessary in order to set in its proper light the significance of inflammatory leucocytosis.

It is now apparent that *leucocytosis represents Nature's attempt to rid the blood and the system, by means of leucocytes and their products, of the bacterial and toxic causes of disease.*

It is now possible, also, to understand the varying significance of hypoleucocytosis, and to place correct interpretations on the various grades of hyperleucocytosis in infectious diseases; to trace the relation between local and intravascular leucocytosis, and explain many of the phenomena of exudative inflammation; to properly interpret the predominance of mononuclear, or of polynuclear, and, to some extent, of eosinophile cells, and to recognize the specific quality of the different varieties of leucocytes; while the application of all these facts to questions in general pathology has been most extensive.

Ehrlich's Theories of Immunity.

The main contributions which have culminated in the partial acceptance of certain principles governing immunity now known as Ehrlich's side-chain theory have been:

(*a*) The observation by Ehrlich that the neutralization of toxin by antitoxin does not follow the laws of fixed proportions. For example, if to a mixture containing 100 lethal doses of diphtheria toxin neutralized by antitoxin one adds several additional lethal doses this mixture still fails to kill the test animal (Ehrlich's phenomenon).

(*b*) The observation by Bordet that hemolytic serum is inactivated by heat (55° to 65° C.) and may be reactivated by addition of normal fresh serum (demonstration of the "complement").

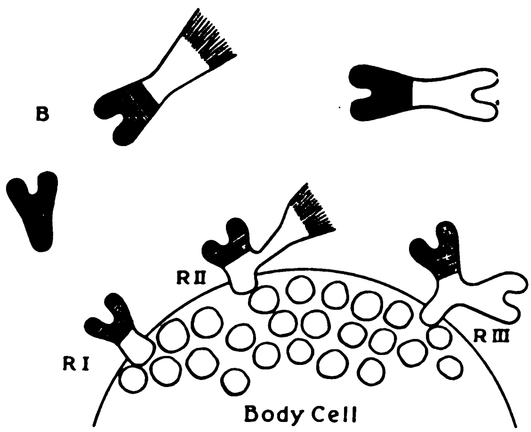
(*c*) The observation by Kraus that bactericidal sera precipitate albumins of bacterial cultures in addition to the agglutination and solution of the bacteria.

These observations taken with what was previously known regarding the bactericidal action of blood serum and body fluids, and regarding agglutination of bacteria by specific sera, the phenomenon of Pfeiffer, and the behavior of leucocytes in infection, all extensively elaborated by a multitude of workers, have led to the present complex state of our knowledge of immunity. The full details of this work are at present most fully presented by Aschoff.

Simultaneously with this line of progress it should not be forgotten that physiological chemistry proceeding along more or less established paths of the chemistry of intracellular ferments, and physical chemistry with its contributions on osmosis and electrolytic dissociation, have built up a considerable fabric which may in some respects threaten the permanency of Ehrlich's theories. It is not to be supposed that there is only one way in which foreign cells and bacteria or their products can be destroyed *in vitro* or in the body.

The reader who desires to follow the progress of studies on immunity requires a considerable knowledge of Ehrlich's theories and of the technical terms used by this school of workers. The following brief definitions and elementary explanations may temporarily suffice for the purpose:

FIG. 27.



Types of receptors. R I-Order I, including antitoxins, anticomplements, antiamboceptors. antiprecipitins, etc. R II-Order II., including toxins, complements, agglutinins, precipitins. R III-Order III., cytolysins, bacteriolysins, hemolysins, etc. B, receptors free from cell, called *haptins*.

General Nature of Ehrlich's Theories. Ehrlich believes that the action upon foreign cells exerted by the various principles concerned in immunity is much like the absorption of food products by the cells. No toxic agent can affect a cell unless that cell possesses certain groups of molecules called *receptors*, of which most cells possess several varieties, with chemical affinities for the toxin.

Moreover, when one of these receptors is occupied by a toxic molecule it is rendered useless to the cell and is soon replaced by new receptors of the same type, which develop in excess of the original requirements of the cell and tend to be thrown off as free receptors (haptins).

From the study of the complex processes occurring in the reactions of immunity Ehrlich concludes that these cell receptors are of three types, designated as receptors of Orders I., II., III. (Fig. 27).

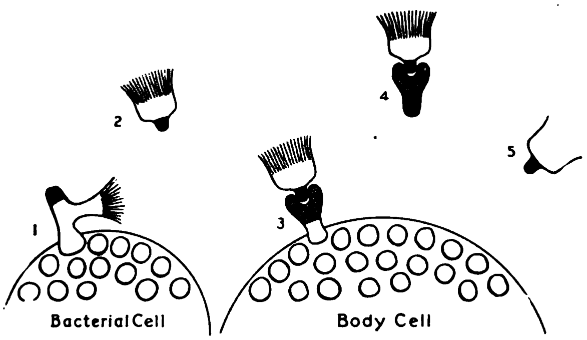
The simplest receptors, Order I., are the antitoxins, which consist of haptins possessing merely one bond of union which unites with the toxin.

Other receptors, Order II., possess two special groups, a haptophore group uniting with the susceptible cell and a toxophore group producing some special action upon the affected molecule or foreign cell. The agglutinins and precipitins are such bodies.

Receptors of Order III. possess two bonds of union, one a haptophore group uniting with the cell, and a complementophore group uniting with the complement (described later) which is necessary to complete the action of this group of principles. The cytolysins belong to this order.

Toxins. The toxins are bodies, like receptors of Order II., possessing a stable haptophore group uniting with the receptors of suitable cells and a toxophore group which exerts the specific poisonous action upon the cell.

FIG. 28.



Development and action of toxins, agglutinins, precipitins. 1. Development from bacterial cell. 2. Free toxin, etc. 3. United to receptor of susceptible cell and active. 4. United to antitoxin and inert. 5. Toxophore group destroyed, as in the toxoid.

Toxins are composed of various toxin molecules with varying degrees of affinity for antitoxin and various degrees of toxicity. When such toxins, in bulk, are incompletely saturated with antitoxin, the most active toxin molecules are first saturated, leaving less active molecules untouched. Such incompletely saturated toxins are called *toxones* and produce in animals modified toxic symptoms (late pareses in diphtheria). Bordet, however, offers another explanation of the nature of toxones.

Toxins may, spontaneously or from a variety of procedures, lose many or all of their toxophore groups, without losing their haptophore groups or their capacity to unite with antitoxin. They are then called *toxoids* (Fig. 28).

A toxin cannot unite with a cell which does not possess receptors with affinities for that toxin. Although most cells possess some receptors for all toxins, some cells have a great abundance of receptors for particular toxins. Thus, brain tissue of many animals absorbs many times as much tetanus toxin as does liver tissue.

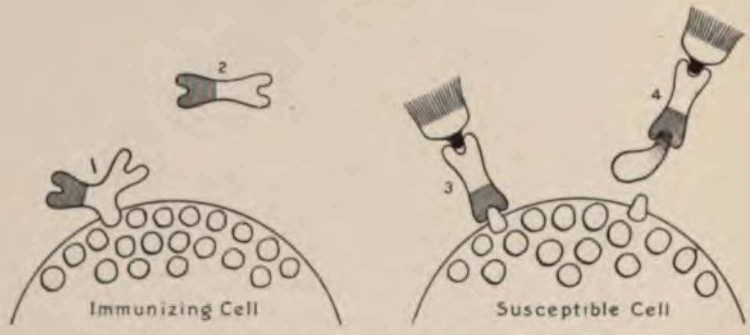
The mode of action of the toxophore group upon the cell is not understood, but resembles in many respects a fermentative process. All these combinations and actions require time.

The haptophore group of toxins is usually unaffected by moderate heat, but the toxophore group, while not thermolabile, is less stable than the other and may be destroyed by many procedures not affecting the haptophore group. Immunization by toxones and toxoids, which possess unsaturated haptophore groups, has been demonstrated.

Cytolysins, Hemolysins, Cytases. The destruction of bacterial and other foreign cells results from the interaction of two principles, the *immune body* (*amboceptor, fixator, substance sensibitatrice*) and the *complement* (*alexine, addiment*).

The complement is derived principally from leucocytes, is present in all normal sera, and is destroyed by heating to 55° or 65° C. for one-half hour. There are, perhaps, thermostable complements. The complements, like receptors of Order II., possess two groups, a haptophore group uniting with the immune body, and a toxophore group exerting destructive action on the attacked cell. Heat destroys the toxophore group, and heated complements are called *complementoids*. They may still combine with the immune body by their uninjured haptophore group. Although complement is present in normal serum, Ehrlich claims the existence of a multiplicity of complements in both normal and immune sera. Complements are less specific in their action than immune bodies. Thus the immune body of humanized rabbit serum—*i. e.*, one which has been immunized to human blood—acts almost exclusively on human red cells, but the complement for such immune body may be supplied by a variety of sera. Yet for bacterial immune bodies some complements are much more active than others and some are entirely inert. The amboceptor (immune body) of cytolytic sera is derived from the cells against which the immunization is directed and from the leucocytes, and consists of the free receptors of these cells, receptors of Order III. For example,

Fig. 29.

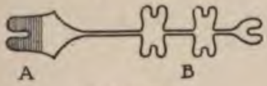


Development and action of cytolytic agents. 1. Amboceptor developing from immunizing cell. 2. Free amboceptor as in hemolytic serum. 3. Complement anchored to cell by amboceptor. 4. Complement anchored to amboceptor, but prevented from action on susceptible cell by antiamboceptor.

the amboceptors of humanized rabbit serum consist of free receptors produced in excess and cast off into the serum from the red cells of the rabbit affected by the toxic influence of the injected human red cells. They are also produced by other cells which have to some extent the same receptors as the red cells. The amboceptors developed in bactericidal serum are derived from tissue cells and especially from the polynuclear leucocytes (Metchnikoff). The amboceptor resists heating above 55° to 65° C. It possesses a cytophile group which unites with the cell, and complementophile groups which unite with the complements. It may be represented by an arrow, the head being the haptophore group and the tail lines the various complementophile groups (Fig. 30).

The function of the amboceptor is, therefore, to fasten the active complement to the cell.

FIG. 30.



Amboceptor.

Agglutinins. When foreign cells or bacteria are injected into suitable animals their sera develop the property of agglutinating *in vitro* the particular cells or bacterium used in the injection. This action resides in an albuminous element of the serum, consisting of the free receptors of Order II. thrown off from the cells chiefly affected by the injections. These receptors differ from those constituting the hemolytic amboceptor. They are thermostabile. They possess a haptophore group uniting with the cell or bacterium and an agglutinophore or zymophore group which brings about this agglutination. The agglutinophore group may be destroyed while the haptophore group persists (agglutinoids). No complement is necessary for their action (Fig. 28).

The nature of the agglutinating process is very obscure.

Precipitins, Coagulins. When defibrinated blood or any albumin in solution is injected into suitable animals their sera develop the capacity of precipitating *in vitro* the albumins used in the injections. The reaction is highly specific (cf. Serum Blood Test). The same laws which govern the agglutinins apply to the precipitins.

Antitoxins, Antiagglutinins, Anticomplements, Antiamboceptors, Anticytolysins. The injection into suitable animals of toxins, agglutinins, complements, amboceptors, or cytolysins gives origin to bodies in the serum which annul *in vitro* the properties of the principles injected. These bodies are receptors of Order I.

The antitoxins are free receptors which unite with the haptophore group of the toxins and prevent the union of the toxin with the susceptible cell. Antiagglutinins and anticomplements are developed in the same manner.

The injection of heated cytolytic serum, free from complement, produces an antiamboceptor which unites with the cytophile group of the amboceptor, thus preventing its union with the susceptible cell, but leaving the complementophile group still capable of attracting the complement.

The injection of a bactericidal or cytolytic serum containing both amboceptor and complement, produces both antiamboceptor and anticomplement (Fig. 29).

Clinical Types of Leucocytosis.

Digestion Leucocytosis. Although digestion leucocytosis was one of the earliest of observed phenomena regarding leucocytes (Nasse, Virchow), owing to the uncertain methods employed by some later observers, and to the subsequent discovery of other marked physiological variations in the numbers of the cells, the occurrence of a leucocytosis referable to digestion was not fully accepted until Pohl, in dogs, and Limbeck and R. Müller, and Rieder, in man, investigated the subject with the necessary precautions against possible disturbing factors.

It is now evident that in order to demonstrate digestion leucocytosis it is necessary to know the normal percentage of each individual, and not to rely upon general averages, as the effect of digestion is not great, and the leucocytes at periods well distant from meals vary considerably according to the health, age, state of circulation, nutrition, and digestion, of the individual. With these precautions it has been found that a full meal raises the number of leucocytes in the average healthy subject about 33 per cent. (Rieder), beginning certainly within one hour, reaching a maximum in three to four hours, and thereafter gradually declining. The exact figure reached cannot have much significance, for the reasons above stated, but in adults the maximum is not often above 15,000 to 16,000. In healthy fasting subjects the increase is rarely over 3000 cells, but in invalids the variations are slightly greater.

A failure of digestion leucocytosis in healthy adults is sometimes observed and may be referred to a prolongation of the process, or to chronic torpidity of the intestines, or to accidental causes.

The quality of the food has sometimes a distinct effect on the grade of leucocytosis. Highly albuminous meats, readily digested and absorbed in considerable quantity, have much more influence upon leucocytes than a meal of vegetables and fats. Indeed, although Duperie has reported digestion leucocytosis after exclusively vegetable diet, it is usually not seen in any vegetarian animal, probably because of slower digestion and absorption.

Of the disorders in which digestion leucocytosis may be expected to fail the mildest are those marked by torpidity of stomach and bowels, which lengthens the time and diminishes the completeness of digestion and absorption (Rieder). R. Müller found it difficult to excite leucocytosis in *anemic subjects*, except by very large meals, until the general condition of the patient was much improved, and Rieder also observed no increase but even a diminution of white cells in similar cases. Among various hospital cases confined to bed from chronic complaints the leucocytosis sometimes exceeded the normal limits, sometimes was absent.

When from any other cause the leucocytes are distinctly increased, as in pregnancy, inflammatory disease, etc., digestion usually fails to produce a further demonstrable increase.

In children, up to fifteen years of age, most observers have found more marked digestion leucocytosis than in adults, especially after a meat diet (v. Jaksch, Rieder). The first digestive activity in the newborn infant excites a considerable leucocytosis, Schiff finding an increase from 19,000 to 27,000 one hour after the first nursing, and 36,000 at the end of forty-eight hours, after several nursings. *Between the tenth and fifteenth years* Rieder found leucocytoses of 3000 to 9000, the average increase being greater than in adults. In the aged it is somewhat less than in adults (Pohl).

In carcinoma of the stomach, R. Müller first called attention to the absence of distinct digestion leucocytosis which marks this condition, finding that without regard to the presence of HCl or pepsin these cases do not show a pronounced increase of leucocytes after meals. From later reports it appears that about 90 per cent. of cases of gastric cancer fail to show any distinct digestion leucocytosis (2000 to 3000), although there are some clear exceptions to the rule, and more examples of a slight increase. This failure of leucocytosis is apparently independent of the presence of HCl or pepsin, occurs when stenosis does not exist, and is referred by Schneyer to involvement of the neighboring lymphatics, and to complicating gastric catarrh. In very advanced cases it is perhaps invariably absent (Hartung). In various other conditions sometimes simulating gastric cancer, as benign stenosis, ulcer of stomach, chronic gastric catarrh (Schneyer, Capps, Cabot), as well as in carcinoma of other viscera (Hartung), well-marked digestion leucocytosis is the rule.

As a diagnostic test, the absence of digestion leucocytosis has not been found to give as reliable evidence as was first hoped, because, as recently shown by Sailer and others, it is too frequently absent in other conditions. As evidence against cancer, the presence of digestion leucocytosis is, however, of considerably greater value, though by no means positive. Nevertheless the examination of the blood deserves a place in the diagnosis of this often obscure disease.

Origin of Digestion Leucocytosis. The essential factor in digestion leucocytosis is, without doubt, the absorption of a considerable quantity of albuminous principles. This absorptive process has been shown by Hoffmeister to excite a considerable proliferation of mononuclear cells in the adenoid tissue throughout the gastro-intestinal tract. It would seem that such increase of mononuclear cells must necessarily cause an increased number of these cells to reach the circulation, and Pohl, in fact, reported finding the mesenteric veins during digestion in dogs, much richer in leucocytes, both mononuclear and polynuclear, than the arteries. Rieder, however, could not find such a disproportion between veins and arteries, and in the absence of other experimental data it becomes necessary to suppose that the marked increase of lymphocytes which characterizes digestion leucocytosis is referable to an increased outpour of lymph from the thoracic duct, the occurrence of which during digestion there are obvious reasons for accepting.

Since the proportions of mononuclear and polynuclear cells are not greatly disturbed in digestion leucocytosis, the marrow must furnish a considerable number of neutrophile leucocytes, and the chemotactic properties of the absorbed albumins must be regarded as the chief cause of their outpour.

Regarding the exact proportions of different cells in digestion leucocytosis data are not numerous but sufficient to show that this is a "mixed leucocytosis," both lymphocytes and polynuclear cells being increased, more especially the lymphocytes. The eosinophiles are usually reduced (Rieder).

Leucocytosis after Muscular Exertion and from Perspiration. In each of four contestants in a twenty-five mile running race, Larrabee found leucocytosis, 14,400 to 22,200 cells, and a few myelocytes. The subjects lost, during the race, from two and a half to five and a quarter pounds in weight, and were greatly exhausted. This leucocytosis is probably of similar significance with that observed after convulsions.

In twenty-nine children subject to spontaneous sweats, or made to sweat by hot-air baths, Hannes found leucocytosis in 77 per cent. In seven cases there was an increase of 3000 cells; in 15, an increase of 3000 to 5000. The leucocytosis disappeared within one-half hour.

Leucocytosis of Pregnancy. The occurrence of moderate leucocytosis in the latter months of pregnancy, though previously known, was first fully studied by Halla, who found a marked increase in ten, a slight increase in six (12,000 to 13,000), and no change in three, out of nineteen cases examined.

Rieder eliminated any possible effects of digestion, allowing his patients to fast sixteen hours, and found a leucocytosis, averaging 13,000 cells, in only twenty-one of thirty-one cases, all the exceptions being multiparæ. Of seventeen multiparæ leucocytosis was absent in ten. Limbeck found 11,000 to 13,000 cells in each of four cases examined, and Cabot reports unusually high counts (25,000 to 37,000) in three, and the ordinary increase in nine cases. Hubbard and White report polynuclear leucocytosis in 80 per cent. of their fifty-five cases, most marked and constant in young primiparæ. The averages twenty-four hours before labor were: primiparæ 15,000, multiparæ 11,700. Before the end of the third month no leucocytosis is to be expected (Rieder), but the exact period of its appearance has not been determined. Rieder made the interesting observation in six cases that in the ninth month of first or subsequent pregnancies, whether leucocytosis exists or not, digestion causes a diminution instead of an increase of white cells.

After parturition, the leucocytes gradually diminish, reaching the normal usually in four to fourteen days, but this diminution is frequently interrupted by slight disturbances referable to the repair of marked erosions or tears of the genital tract, to mild disorders of the breasts, and to the loss of blood. During and immediately after childbirth a considerable increase of leucocytes has been observed in a moderate number of reported cases (Kosina and Eckert, Malassez, Fouassier, Rieder).

The studies of Zangemeister and Wagner have important bearing on previous work in this field. They found that among healthy women between twenty-one and thirty-four years of age the leucocytes varied greatly, and in sixteen there were over 12,500 white cells. In fifty-seven pregnant women about the same figures and variations were obtained, except that counts below 10,000 disappeared, and twenty-one gave over 12,500. Parturition increased the leucocytes in all of sixty-three cases up to the birth of the child, when the count promptly fell. In childbed, seventy-five cases, there was usually a steady decrease, sometimes interrupted by after-pains, absorption of lochia, etc.

The leucocytosis of pregnancy has been referred to various causes, none of which seem to meet all the requirements. Virchow found a parallel between the increase of leucocytes and the widening of the uterine and abdominal lymphatic vessels and nodes and the increase of metabolism in the uterus and its contents. Mochnatscheff's comparisons of the numbers of leucocytes in the finger blood and in the *cervix uteri* do not seem calculated to demonstrate an increased supply of any variety of cell from the latter region. Limbeck suggests that the changes in the breasts are the chief factor, recalling the round-cell infiltration which many such glands show, as an indication of very active cellular processes. He, with others, has supposed that this leucocytosis, like that of the newborn, may represent a continuous effect of digestion, an opinion which cannot be accepted, since digestion leucocytosis is usually suppressed in late pregnancy. Considering the behavior of leucocytes in general, it seems hardly a matter of surprise that the active cellular processes in the breasts, uterus, vascular system, and fetus, and the associated increase of metabolism should, when instituted for the first time, find a sympathetic excitement in the blood-producing organs. Being a "mixed leucocytosis," with all but eosinophiles normally represented, this leucocytosis is probably not either inflammatory or toxic.

The Leucocytosis of the Newborn. That the blood of newborn infants invariably shows a well-marked leucocytosis was noted by many early observers, in greatest detail by Hayem, who found an average at birth of 16,000 to 18,000, a rapid decrease to 7000 toward the third to fourth days, when the initial loss of weight becomes most marked, and a subsequent increase to 9000 or 11,000, with beginning increase in body weight. In children at eight months he found 14,000 to 21,000 white cells, while by the fifteenth month the numbers commonly dropped to 10,000, and he noted also that the increase was largely in the nature of a lymphocytosis, the small lymphocytes being four to five times as numerous as in adults.

These main features of the leucocytosis of the newborn appear again in Rieder's observations, who found slightly greater numbers, 14,200 to 27,400, in a few subjects, in whom the initial decrease on the third to fifth days was also well marked and apparently unaffected by the first demands on digestion. Rieder found the leucocytosis of the child uniformly greater than that of the mother, and, except for the preliminary decrease of the first week, much more persistent. His differential counts show that the lymphocytes are not in excessive proportion at birth, but that this excess becomes established later. (So also Gundobin.) His cases showed at birth a considerable excess, also, of eosinophile cells.

Schiff examined eleven infants twice daily for two weeks, his results showing *excessive variations* in the numbers of leucocytes in the first few days (10,000 to 36,000), referable apparently to overfeeding and diarrhea. The preliminary decrease beginning on the third to fifth days is quite noticeable in his tables. At the end of two weeks the leucocytes numbered 10,000 to 15,000.

Gundobin's observations show that the leucocytes remain high during the first year of infancy (11,000 to 14,000); that the excess of lymphocytes is established before the tenth day and persists throughout the first two years (50 to 65 per cent), when they begin to decline, reaching the proportions of the adult about the eighth to tenth years.

OBSERVATIONS ON THE LEUCOCYTOSIS OF THE NEWBORN.

Hayem	average for forty-eight hours,	18,000
"	third to fourth day,	7,000
"	after fifth day,	9,000 to 11,000
Otto	ten to twenty-five hours,	23,000 to 25,000
Schiff	forty-eight hours	10,000 to 32,800
"	fourth to eighteenth day,	12,000 to 13,000
Woino-Oransky	at birth,	16,980
"	fourteen hours,	20,980
"	second day,	31,680
Gundobin	last day, fetus,	8,053
"	at birth,	19,600
"	twenty-four hours,	23,000
"	forty-eight hours,	17,500
Kruger	one to three days,	18,000
"	after third day,	15,000
Rieder	at birth,	14,200 to 27,400
"	second to fourth day	8,700 to 12,400
"	after fourth day,	12,400 to 14,800

From the above conflicting reports it is difficult to give precise details regarding the leucocytosis of the newborn. Rieder's observations seem to the writer the most reliable, in which a moderately high proportion of white cells is found at birth, a distinct decrease on the second to fourth days with the diminution of weight, followed by a less marked but persistent leucocytosis. Further very careful studies are needed in this field.

Regarding the origin of this form of leucocytosis, opinions are at variance. That the initial increase is referable to concentration of the blood and venous stasis seems to be true both of red and of white cells. The preliminary decrease the writer believes to be due to improvement in the venous circulation with relief of cyanosis, and the absorption of fluids. The subsequent increase and permanent leucocytosis of infancy, being largely a lymphocytosis, probably represents to a considerable extent continuous digestion leucocytosis.

That it is not entirely referable to digestion is indicated by the low counts found by Rieder on the second to fourth days, after frequent nursing.

Post-hemorrhagic Leucocytosis. A well-marked leucocytosis following acute hemorrhage was described by the older writers (Virchow, Nasse, etc.), but the detailed study of this condition was reserved until Lyon and Huhnerfauth investigated the effects of hemorrhage by the newer methods.

Huhnerfauth found in nine dogs, losing about 4 per cent. of their body weight by acute hemorrhage, that the leucocytes were often slightly diminished immediately after the operation, much increased (maximum 45,000) on the following day, and that this increase persisted in some degree for two to three weeks. Lyon, in similar experiments, also found an initial decrease a few minutes after the hemorrhage, but soon a very marked increase of the white cells, reaching its highest point (62,000) within six to eight hours, declining rather rapidly after three to four days, but persisting thereafter in moderate degree for days or weeks. In the human subject after a surgical operation attended with dangerous hemorrhage, Lyon found, after one hour, 41,625 leucocytes; after five days, 14,300, followed by a slight increase for one week. In a case of leukemia, splenectomy caused the leucocytes to jump from 463,000 to 850,000 several hours before death from hemorrhage and shock. Rieder repeated the experiments on dogs, finding the usual changes, as a rule, but in one instance a large hemorrhage failed to cause leucocytosis. In differential counts he observed that most of the leucocytes were polynuclear, finding as high as 97 per cent. of neutrophile cells immediately after the hemorrhage. In the human subject he was unable to find very pure examples of post-hemorrhagic leucocytosis, but reported 15,000 cells after pulmonary hemorrhage in phthisis; 32,600 (1,300,000 red) after hemorrhage from cancer of the uterus; 26,500 (1,985,000 red) in ulcer of stomach.

Head demonstrated a slight temporary diminution of leucocytes occurring a few minutes after severe hemorrhage in dogs, which was soon replaced by leucocytosis lasting seven days.

The neutrophile cells are usually the ones most affected, the eosinophiles sometimes appearing in considerable numbers (Lyon, Hall and Eubank), and the small lymphocytes showing a relative increase (Rieder). Ehrlich and Lazarus found 13.7 per cent. of myelocytes in a marked post-hemorrhagic leucocytosis, and in one of the writer's cases of splenectomy with profuse hemorrhage the blood for some days closely resembled that of myelogenous leukemia.

In general, the leucocytosis following hemorrhage is in proportion to the extent and rapidity of the loss of blood, but it usually disappears or greatly diminishes long before the red cells are restored. The infusion of fluids has been found to considerably increase the leucocytosis. This result has been repeatedly observed in cases of splenectomy (q. v.).

The not infrequent failure of post-hemorrhagic leucocytosis, as reported by Rieder, Stengel, Cabot, and others, has not been explained. In such cases the remaining cells may be largely lymphocytes (Stengel). Erb, however, reported *marked absolute lymphocytosis* after hemorrhage.

It is generally agreed that the chief factor in the production of post-hemorrhagic leucocytosis is the outpour of lymph which restores the lost bulk of blood. Were this the only factor, however, the new cells ought to be principally lymphocytes, and the high proportion of neutrophile cells commonly seen points to a special draining of the marrow or possibly to increased cellular activity in this tissue. Against this view are the facts that normoblasts are not numerously present until the second to the third day, while the leucocytes are most numerous after a few hours.

The old opinion of Samuel, Virchow, and others, that the more cohesive neutrophile cells are retained in the vessels is perhaps still worthy of consideration. After the infusion of salt solution the high percentage of polynuclear cells can be partly referred to the chemotactic influence of this fluid upon the marrow.

Cachectic Leucocytosis. Of the varieties of leucocytosis classified by Rieder that which is frequently associated with severe anemia and cachexia is the least homogeneous. In its causation figure many of the conditions already shown to frequently excite leucocytosis, especially loss of blood and inflammatory processes. Yet there exists a considerable group of cases, marked by continuous leucocytosis, in which other influences combine to increase the production of white cells, and to develop a somewhat peculiar form of leucocytosis which deserves a special description.

White, Cruveilhier, Paget, and others before them, who first described cachectic leucocytosis, regarded the excess of cells as fragments of the neoplasm. Andral and Gavaret, Chaillou, and Vidal, recognized these cells as leucocytes. This correct interpretation was soon followed by the more detailed study of the condition by Lucke, Sappey, Nepveu, and especially by Hayem, Alexander, Schneider, Pee, Reinbach, and Rieder, each of whom have contributed largely to the present knowledge.

Some of the clearest examples of this sort of leucocytosis are seen in the cachexia of malignant tumors, where the combination of hydremia, local inflammatory processes, and specific toxemia are the principal factors concerned.

Chronic anemia may lead to increase of leucocytes in the circulation through the watery condition of the blood, the increased activity of red marrow, here often moderately hyperplastic, and the lowered blood pressure. Illustrations of the working of these influences are seen in the increase of post-hemorrhagic leucocytosis after infusion, and in the periodical appearance of normoblasts and mixed leucocytosis in advanced chlorotic anemia. Yet chronic hyperemia and extreme hyperplasia of the red marrow alone are not necessarily accompanied by leucocytosis, but often exist with diminished leucocytes, as is usually seen in uncomplicated primary pernicious anemia, so that it is impossible to ascribe to chronic hydremia more than a predisposing influence in cachectic leucocytosis.

Horbaczewski and Lowit[3] ascribe both the hydremia and the leucocytosis of cachexia to the *lymphogogic* and *chemotactic influence of nucleins,* which in increased quantity are set free by the excessive destruction of tissue elements, but Lowit claims from his experimental study of the subject *that increased flow of lymph alone is incapable of producing leucocytosis.*

Local inflammations are probably the most frequent causes of marked cachectic leucocytosis. These may be traced in the growing edges or ulcerating surfaces of malignant tumors, in various internal suppurative or necrotic processes in syphilis and tuberculosis, and in exacerbations of underlying chronic inflammations.

The special involvement of the blood-forming organs by neoplasms and inflammations has been found not only to increase the leucocytes in the circulation, but at times to give a special character to the leucocytosis which may be of diagnostic service—*e. g.*, eosinophilia and lymphocytosis with sarcoma of bone or lymph nodes. While sarcoma is usually attended with lymphocytosis, carcinoma, on the other hand, usually excites a polynuclear leucocytosis, a difference which may perhaps be referred to the freedom of the lymph paths in the former and their occlusion in the latter case.

Finally, there is to be considered as the essential element in cachectic leucocytosis a *chronic toxemia,* which, in several conditions, is of more or less specific character.

Some importance here attaches to the observations of Hayem, who noted the disappearance of persistent leucocytosis (10,000 to 21,000) in four cases after removal of cancers of the breast, while a recurrence of the tumor he claims to have predicted from a slight persistent increase of leucocytes. Grawitz has shown that injections of extract of carcinomatous tissue and of tuberculin have a marked lymphogogic effect. The distinct cachexia which often foreshadows the appearance of a malignant neoplasm is abundant clinical evidence that these new-growths elaborate powerful toxins, but it has never been conculsively shown that these toxins have any particular influence upon leucocytosis, which in the majority of cases seems to arise from other factors. The same conclusion must be drawn from the absence of any uniform relation between cachectic leucocytosis and the extent of miliary tubercles and gummata, or the extent, location, and histological structure of malignant tumors. General miliary tuberculosis, acute or chronic, usually fails to excite leucocytosis. The writer has seen severe anemia without leucocytosis, with extensive gummata in liver, spleen, and thoracic and abdominal lymph nodes, and in general carcinomatosis.

Indeed, in a review of many reported cases and in the writer's own experience, *in the great majority of cases of tertiary syphilis, tuberculosis, nephritis, in a large proportion of carcinomata, and in a rather smaller proportion of sarcomata, cachexia is unaccompanied by leucocytosis unless there is distinct local inflammation, necrosis, or hemorrhage.* Whence it may be stated as a general rule that *marked leucocytosis in the course of cachexia suggests a search for one of these complications.*

Antemortem Leucocytosis. In 1883 Litten reported from several hunded examinations of the blood of forty moribund cases in whom no leucocytosis had previously existed, that he had found in many a more or less pronounced increase of white cells, beginning at a variable period before death.

When dissolution was rapid no leucocytosis was found, but when prolonged, the increase of white cells was very marked (one to five of red). Litten was unable to offer a plausible explanation of the phenomenon and failed to give exact numerical estimates and important clinical details of his cases, so that his interesting observations attracted but little notice.

In 1886, Gottlieb found 30,000 leucocytes six hours before death in a case of pernicious anemia which had previously shown no increase. Rieder found well-marked antemortem leucocytosis in three of four cases, in one of which 89.5 per cent. of 60,000 cells were polynuclear. The case not showing leucocytosis was complicated with aspiration pneumonia. Many later reports of isolated observations on this variety of leucocytosis have proven the frequency of its occurrence, but have not added greatly to the knowledge of its special peculiarities or its significance.

It appears most probable that a considerable variety of factors is concerned in the antemortem increase of leucocytes. Cohnheim's theory that diminishing blood pressure causes an increased outpour of lymph is supported by the occurrence of post-hemorrhagic leucocytosis, but not by the high percentage of polynuclear cells and of nucleated red cells usually present. Litten's suggestion that there is an unequal distribution of leucocytes in favor of the peripheral capillaries is in accord with the known effects of stasis. Limbeck's opinion that antemortem leucocytosis probably results in most cases from terminal infections may certainly apply to many instances marked by excess of polynuclear cells, but not to all, especially to those rare cases showing lymphocytosis.

The character of antemortem leucocytosis seems to depend largely upon the precedent condition. In leukemia and inflammatory diseases the cells principally increased are the polynuclear forms of the marrow, and along with these many nucleated red cells and some myelocytes are commonly drawn into the circulation. In the terminal stages of lymphatic leukemia the lymphocytes may be greatly increased.

Pronounced antemortem lymphocytosis has been seen in pernicious anemia by Cabot and by the writer, in diphtheria and other conditions in children, in typhoid fever, and to a less extent in malaria. Its rather frequent occurrence is constantly leading to erroneous diagnoses of acute lymphatic leukemia.

It would seem that the effects of terminal inflammations, antemortem dissemination of intestinal and other bacteria, antemortem hyperpyrexia, vasomotor paresis, serous exudates, diarrhea, lack of fluids ordinarily ingested, etc., have not received sufficient attention in this important field, and that the entire subject needs investigation on a much more elaborate scale than has yet been attempted.

Experimental Leucocytosis. 1. **By Drugs and Chemicals.** The earliest observations on experimental leucocytosis were those of Hirt, who, in 1856, reported an increase (100 to 300 per cent.) of white cells following the administration of tr. ferri pom., tr. myrrh., etc. The same effect was noted by Meyer from turpentine, camphor, and oil of peppermint. Later Pohl, in the endeavor to explain the origin of digestion leucocytosis, tested the effects of a large number of drugs, finding that the aromatic extracts and oils, vegetable bitters, certain alkaloids (piperin, strychnine, and others) caused a rather distinct (40 to 120 per cent.) leucocytosis, when administered to fasting dogs. Alcohols, salts of various alkalies, lead acetate, cupric sulphate, calomel, caffeine, and quinine he found to be inert, and oxide of iron usually so. In general these leucocytoses appeared within one-half hour and disappeared in two hours, and were therefore less marked and persistent than those excited by the absorption of digested albumins.

Repeating these experiments on man, Binz and Limbeck induced leucocytosis by means of camphor and oils of cinnamon, peppermint, and anise, but Rieder found it impossible to secure much effect from extract of gentian or tincture of myrrh. Even such a bland fluid as solution of common salt in subcutaneous injection has been shown to excite transitory leucocytosis (Lowit, Rieder).

Bernard, and Meyer and Seegen found the leucocytes doubled after the administration of 20 gt. of ether.

Horbaczewski[2] found a moderate diminution of leucocytes in man after administraion of quinine and of atropine, and a considerable increase from antipyrin, antifebrin, and pilocarpine. The writer has failed entirely to produce distinct leucocytosis in rabbits by use of pilocarpine. Gazza reports that digitalis in therapeutic doses causes leucocytosis which increases until death if the drug is pushed to fatal doses.

Winternitz studied the effects of a great variety of drugs, with special reference to the relation between the grade of local inflammation and the degree of intravascular leucocytosis. He divided these agents into two classes, one of which, including salts and simple irritants such as free acids and alkalies, by subcutaneous injection, induced slight local disturbance and moderate leucocytosis and fever; while the other, including vesicants, sapotoxin, digitoxin, silver nitrate, copper sulphate, mercurials, and antimonials, produced aseptic suppuration and higher leucocytosis. He, therefore, established for these agents the same relation between local reaction and general leucocytosis that Limbeck has shown for bacterial cultures.

Moderate grades of leucocytosis have been reported by Wilkinson from the administration of potassium iodide, quinine, salicin, etc., from nuclein by v. Jaksch, from salicylic acid by Schreiber and Zandy, from morphine and sodium salicylate by Bohland. The writer once counted 28,000 cells, mostly polynuclear, in coma from morphine, forty-eight hours before death. All of these observations point to the necessity of considering the effects of drugs in the clinical study of leucocytosis in disease.

2. **By Bacterial Cultures.** Limbeck injected cultures of bacteria into the knee-joints of fasting dogs, and found the maximum leucocytosis at the end of six to twenty-four hours, two to three times the normal numbers being commonly reached, and 88 to 93 per cent. of the cells being polynuclear. The pyogenic staphylococci were most active, increasing the leucocytes sixfold to sevenfold; *streptococcus pyogenes* came next, and Friedlander's *pneumobacillus* third, in effectiveness.

Rieder repeated these experiments, using cultures from solid media only. He showed that such material was very much less active in producing leucocytosis than are the fluid cultures, that the increase is preceded by a transient decrease of cells, and that the injections may be followed by persistent hypoleucocytosis and death of the animal.

A sufficient number of observations have since shown that practically all pathogenic bacteria in subcutaneous or intravenous injection exert positive chemotactic influence upon leucocytes. The duration of the period of diminution and the degree of subsequent increase of leucocytes vary considerably with the different species, cultures, and individuals.

3. **By Bacterial Proteins, etc.** After Limbeck's demonstration of the chemotactic effects of bacterial cultures, it was soon shown that various bacterial and other extracts are equally powerful excitants of leucocytosis, as cadavarin, putrescin, ptomaines of decomposing flesh, extracts of sterilized cultures of staphylococci, and phlogosin, a crystalline alkaloid obtained from similar cultures (Scheurlen, Grawitz,[2] Behring,[2] Arloing, Leber[1]).

The more complete separation of the active chemotactic principles of bacterial cultures was accomplished by Buchner following Nencki's method. Buchner found that the leucocytes are influenced solely by the albuminous principles of bacterial cultures, and these he isolated from a considerable number of species, in the form of bacterial "proteins." From *bacillus pyocyaneus* he secured a protein which, after four daily doses of two grammes, increased the leucocytes sevenfold. Testing the effects of the decomposition-products of animal tissue, peptone, alakli-albumin, and calcium, he found these agents comparatively inactive and concluded that the principles derived from inflamed tissue are unimportant adjuncts to the bacterial proteins in exciting inflammatory leucocytosis.

Koch's tuberculin was early shown to cause at the height of the reaction a moderate and rather transitory increase of polynuclear leucocytes and a simultaneous diminution of eosinophile cells (Uskow, Tschistovitch, Zappert). Botkin found it impossible to increase the effects by repeated daily doses. A marked and persistent increase of eosinophile cells, reaching in one case 85 per cent. and in another continuing for ten weeks, following the febrile reaction and frequently associated with cutaneous eruptions, has been noted by Grawitz,[3] Neusser,[2] Canon, Botkin,[2] and Zappert.

A recent extensive study by Liebmann indicates that tuberculin influences both leucocytes and the tubercle bacillus, drawing each into the circulation, in man and lower animals. Liebmann could find no great uniformity in the behavior of eosinophile cells, but in rabbits and guinea-pigs tuberculin caused a marked increase of neutrophile cells, a moderate increase of mononuclear leucocytes, and the appearance of a moderate number of mast-cells.

That leucocytosis follows the injection of a great variety of bacterial filtrates and extracts has been shown also by a large number of later experiments. The grade of leucocytosis varies considerably, depending on the quantity and virulence of the toxin and the susceptibility of the animal.

Rieder found the leucocytes increased twelvefold after three daily injections of pyocyanin. Occasionally one encounters persistent hypoleucocytosis, as did Romer after using large injections.

4. **By Various Organic Principles.** The Dorpat school first studied the effect upon leucocytes of another group of agents, including several animal principles. They found hypoleucocytosis followed by hyperleucocytosis in marked degree after the injection of fibrin ferment, pepsin, peptone, hemoglobin, decomposing albuminous fluids, pus, and crushed lymphoid tissue (Hirt, Bojanus, Hoffman, Himmelstjerna, Heyl, Groth).

Lowit studied the effects of various albuminous and organic principles, injected into animals, finding hypoleucocytosis followed by varying grades of hyperleucocytosis from hemialbumose, peptone, pepsin, nucleinic acid, nuclein, urea, sodium urate, curare, and pyocyanin, and tuberculin.

Vegetable albumins were first used to excite local purulent exudates by Buchner, who obtained aseptic pus in large quantities by the injection into the pleura of ground wheat, pus, fossil earth, and gluten casein. Rieder and Hahn also secured a high grade of leucocytosis by the same agents.

Goldscheider and Jacob proceeded still further in the study of the chemotactic properties of animal extracts, finding powerful effects from the injection of glycerin extracts of spleen, thymus, and marrow, but none from extracts of thyroid, liver, kidney, and pancreas.

Applications of Experimental Leucocytosis. In 1894 Pawlowsky reported that he had saved animals from fatal doses of anthrax by injections of papayotin, abrin, and ricin, which are very active excitants of leucocytosis, and he recommended that anthrax in man be treated by local injection of these agents. In guinea-pigs and rabbits in which leucocytosis had been excited by papayotin, or better by abrin, injections of tubercle bacilli were less frequently followed by generalization of the tuberculous process, sometimes by greater tendency to fibrous growth in the tubercles, or even by total disappearance of tubercles already existing.

In 1895 Lowy and Richter reported that rabbits in which they had established marked leucocytosis by repeated injections of "spermin" withstood injections of pneumococcus cultures three to four times as large as were required to kill control animals. The therapeutic effect was much less marked when the bacterial injections followed those of spermin within twenty-four hours or less.

Goldscheider and Müller then took occasion to recall some unsuccessful experiments of their own performed on guinea-pigs, in which leucocytosis had been excited by injections of splenic pulp extract. This procedure they had found to yield no therapeutic influence on infections by *bacillus diphtheriæ, tetani, proteus,* or *pneumococcus.*

Goldscheider and Jacob had also previously attempted, without success, to favorably influence by artificial leucocytosis the course of typhoid fever and puerperal sepsis.

In animals which had received injections of albumoses Jacob found that subsequent infection with virulent bacterial cultures was better withstood if the infection occurred when the leucocytes were on the increase from the previous injection of albumose, but when the infection occurred in the stage of hypoleucocytosis it was more rapidly fatal than in the control animals. Considerable therapeutic effect was apparently obtained when the administration of albumose occurred during the increase of leucocytes resulting from the bacterial injection.

Hahn was able to retard the progress of anthrax infection in rabbits by previously exciting leucocytosis by means of albumoses, but with a few favorable results were a larger number of failures. He went on to show that the bactericidal action of blood both in man and animals is increased during the stage of hyperleucocytosis.

From the foregoing studies it is evident that there is a rational basis for the employment of artificial leucocytosis in the treatment of some infectious processes, since both the phagocytic and bactericidal powers of the blood are thereby increased. It has recently been shown that the treatment of some infectious diseases by animal serum is ineffective, probably because the serum, rich in immune body, "amboceptor," is deficient in suitable complements, and various attempts have been made to supply such complements artificially. Yet the results in man are as yet not very favorable, since there is no known method of exciting with impunity continuous leucocytosis in the human subject, and the search among lower animals for complements adapted to the human subject has not thus far succeeded. The limitation of puerperal septicemia by exciting localized abscesses by injections of vesicants, the extrusion of inoperable malignant tumors by erysipelas, and the treatment of typhoid fever with pyocyanin illustrate some of the practical difficulties in the way of this branch of therapeutics.

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THE OCCURRENCE OF EOSINOPHILE CELLS, MAST-CELLS, MYELOCYTES, AND LYMPHOCYTES.

EOSINOPHILIA.

Physiological Variations. The number of eosinophile cells in the blood of healthy adults varies, according to Zappert, between 55 and 784 per cubic millimetre (0.67 to 11 per cent.), but in the majority of individuals the limits are between 1 and 4 per cent. Zappert regards 50 to 100 per cubic millimetre as a low normal count, 100 to 200 as intermediate, 200 to 300 as high normal, and over 300 as pathological.

In children a relatively high proportion of eosinophile cells is the rule, and the variations in number are slightly greater. The average appears to be about 1 to 2 per cent. greater than in adults.

There are no uniform changes referable to old age, sex, pregnancy, menstruation, or digestion. A slight increase after coitus has been observed. According to Horder's observations, eosinophilia is a racial characteristic of the natives of South China. While Europeans in that locality seldom showed more than 5 per cent. of these cells, the healthy Chinese gave an average of 15 per cent., in anemia and rheumatism 20 per cent., malaria 12 to 37 per cent., leprosy 30 to 42 per cent.

Pathological Variations. From the fact that eosinophile cells may be multiplied many times without passing the limits established as physiological, it is obvious that none but considerable changes in their absolute numbers can have any distinct pathological significance; yet the observations on eosinophilia have referred quite as much to persistently high or low averages within normal limits as to distinct increase or decrease, and a certain pathological significance has been shown to go with these lesser variations.

Diseases of the Blood. In *leukemia* the presence of large numbers of eosinophile cells was first regarded by Ehrlich as the pathognomonic sign of myelogenous leukemia, but it has since been shown that eosinophilia may be very marked in other conditions, and that the proportion of these cells in leukemia almost invariably lies within normal limits (1 to 7 per cent.). Their total numbers, however, are very greatly increased, Rieder's highest estimate reaching 29,000 per cubic millimetre. In lymphatic leukemia they are commonly absent.

The presence of eosinophile myelocytes was regarded by Müller and Rieder as pathognomonic of leukemia, but these cells are not infrequently found in other conditions. The writer finds that very large darkly staining eosinophile granules in mononuclear cells are practically limited to leukemic blood, but isolated examples have been seen in malaria (Bignami).

In *chlorosis* the eosinophiles may be moderately increased, normal, or much diminished. Zappert was unable to verify Neusser's belief that their absence in chlorosis is of unfavorable import.

In *secondary anemia* the occurrence of eosinophile cells depends largely upon the underlying condition, the anemia itself not effecting any increase but rather a decrease. There are, however, many examples of secondary anemia with marked eosinophilia—*e. g.*, ankylostomiasis.

Diseases of Lungs. The occurrence of large numbers of eosinophile cells in the blood and sputum of *asthmatics* was first noted by Gollasch, and has been uniformly confirmed by later observers (Leyden, Zappert, Mandybur, Weiss, Gabritschewski), who have found them to vary in the blood of such cases from 9 to 22 per cent. In simple emphysema they are not markedly increased, and in pulmonary tuberculosis they are usually much diminished. Where tuberculosis complicates emphysema, both blood and sputum may, however, show an excess of eosinophile cells (Weiss, Aronson and Philip).

In *tuberculosis* of lungs or other tissues an absence of eosinophile cells from the blood is often observed, and this fact has been of considerable value in differential diagnosis between this and other conditions in which normal or increased numbers of these cells are present; yet when tuberculosis is accompanied by cachexia and irregular suppuration, eosinophile cells may reappear in moderate numbers. Neusser believes that when these cells persist in tuberculosis the outcome is usually favorable, since gouty subjects are comparatively resistant to this infection; yet tuberculosis in emphysematous lungs is not infrequently seen at autopsy. The eosinophilia following injections of tuberculin has already been considered. In subacute or chronic tuberculous lesions eosinophile cells are very frequently present in the tissues in large numbers.

In febrile diseases of **liver and gastro-intestinal tract** the occurrence of eosinophile cells in the blood shows no uniformity. They have been found moderately increased in cirrhosis of the liver.

In **chronic nephritis** the eosinophile cells are apt to reach the higher physiological limits. Neusser believes that uremic seizures are regularly accompanied by increase of these cells in the blood, and Zappert and Castellino report such cases.

In **nervous diseases**, functional and organic, Zappert found, as a rule, a moderate increase of eosinophile cells, without being able to substantiate Neusser's classification of neuroses into those with and those without eosinophilia. The latter he preferred to regard as exceptions to the very general rule. In a distinct sympathetic neurosis they were not increased. In general insanity no uniform increase has been demonstrated (Zappert, Krypiakiewicz). Jelliffe found a moderate increase in one and a total absence of eosinophiles in five of twenty cases of general paresis. In Basedow's disease they are usually increased (Neusser).

Neusser finds eosinophilia in a variety of nervous disorders, the psychoses of menstruation, puerperal mania, epilepsy, tetany, Basedow's disease, hemicrania. In acute mania not connected with pregnancy, and in melancholia, Somers found a well-marked and uniform eosinophilia.

Tumors. From observations on twenty-four cases Zappert concluded that a very uncertain eosinophilia may occur with new-growths, especially when cachexia is not advanced. He found 17.76 per cent. of eosinophiles in one of several cases of lymphosarcoma, and a distinct increase in isolated cases of carcinoma. Neusser claims that eosinophilia arising in the course of a tumor growth indicates metastasis in the marrow, and Reinbach found 60,000 in a case of lymphosarcoma with metastasis in the marrow. Feldbausch studied the occurrence of eosinophile cells in tumors. He always found them in considerable numbers in the growing edges of epitheliomata, less often in carcinomata, while in some lymphosarcomata they were very abundant.

Skin Diseases. Various cutaneous lesions have furnished some of the most marked and interesting examples of eosinophilia. While the highest numbers of these cells have been found in pemphigus (4800), it has been shown by Canon and verified by Zappert that the eosinophiles are affected not so much by special forms of cutaneous lesions as by the extent, intensity, and lack of healing tendency on the part of the lesion. Moreover, it appears that *local afflux* of eosinophile cells occurs in the early and in the less active stages of many cutaneous lesions, and is often insufficient to cause a general increase in the blood. That many lesions are caused by local toxic agents having special chemotactic influence over eosinophile cells is indicated by the fact that in pemphigus artificial blisters do not necessarily contain the larger numbers of these cells that are found in the spontaneous lesions (Kreibich).

Among the diseases showing high eosinophilia may be mentioned: *pemphigus*, *eczema*, *scleroderma*, *psoriasis*, *pellagra*, *lupus*, if widespread, *urticaria* (60 per cent., Lazarus) (Zappert, Neusser, Canon, Tschelenef, Laredde).

In the blood of *leprosy* Gaucher and Bensaude found 8.48 to 28 per cent., Darier as high as 61 per cent., and Bettman 7 to 18.4 per cent.

In pemphigus Coe reports one case with 12,000 leucocytes and 10 to 51 per cent. of eosinophile cells, and another fatal case with 6 per cent. Russ observed a fatal case in which there was no eosinophilia.

In *zoster* Bettman found a high proportion of eosinophile cells in the vesicles, but usually no increase in the circulating blood.

Sabrazes and Mathis followed the changes in the blood in eight cases of zoster. The Hb and red cells were unaffected. On the first day of the eruption they found leucocytosis 11,780 to 16,740, which increased until the third to fifth days, maximum 17,900, and usually persisted three to five days longer, disappearing as the vesicles began to fade. During desquamation, eighth to twelfth days, there was sometimes a second increase (14,880 to 16,120), especially of eosinophile cells. All varieties of white cells were in excess, and the percentage of polynuclear cells was rarely above normal. The eosinophile cells were usually abundant, 4 to 8 per cent., and once reached 20.5 per cent. The vesicles showed progressive increase of polynuclear cells until the sixth day, when eosinophile leucocytes began to appear in the exudate.

Post-febrile Eosinophilia. In nearly all forms of acute polynuclear leucocytosis an absence of eosinophile cells has been noted. Scarlet fever, acute rheumatism, and malaria sometimes furnish notable exceptions to this rule. Early in the decline of the febrile process, and before the neutrophile leucocytes have greatly decreased, eosinophile cells begin to reappear, and may shortly be found in more than normal numbers. The reappearance of eosinophile cells in pneumonia and septic processes, etc., has often been found to herald a favorable turn in the disease, but usually other favorable clinical symptoms are present at the same time.

In *malaria*, eosinophile cells usually persist in small numbers during the paroxysm, while in the afebrile interval, and in chronic cases, a slight eosinophilia exists, and may be a useful diagnostic feature of the blood.

In *acute rheumatism* they are usually present in moderate numbers during the fever, increase slightly during convalescence, and in one of Zappert's cases a relapse was attended with a further increase.

Acute Exanthemata. In *scarlatina* there is a remarkable exception to the usual rule that eosinophiles disappear during febrile leucocytosis, for in this disease they usually persist and may be markedly increased. This peculiarity was first noted by Kotschetkoff, who found that the oxyphile cells steadily increase during the fever, reach a maximum in the second or third weeks (8 to 15 per cent.), and fall to normal in six weeks. In his very severe cases, however, they fell more rapidly. Rille found marked eosinophilia in severe cases, and Zappert, Silvester, and Felsenthal have added confirmatory reports.

In *measles* eosinophiles are usually normal or diminished (Zappert, Felsenthal, Cabot).

In some other acute simple erythemata Zappert found distinct eosinophilia.

Gonorrhea. According to Bettman, eosinophile leucocytes appear in gonorrheal pus during the first few days of the disease when the exudate is largely serous. Here as elsewhere it seems that when the stage of exudation of neutrophile cells is reached the eosinophiles largely or entirely disappear, to return again during the decline of the discharge. This latest contributor also agrees that the involvement of the posterior urethra and prostate is usually attended with a marked increase of oxyphile cells in the discharge, and he finds further a similar influence from the involvement of the epididymis.

Most observers are agreed that the eosinophile cells of the blood are increased when they are abundant in the discharge, but not in any proportionate degree (Posner, Finger, Pezzoli, Janowsky, Epstein, Tscheleneff, Vorbach). Bettman found 25 per cent. in a case complicated by epididymitis.

Syphilis. A very uniform increase of eosinophiles accompanying cutaneous syphilis was noted by Rille and by Loos, but the observations of Müller and Rieder and of Zappert, though less numerous, did not bear out these claims. In a case of congenital syphilis Müller and Rieder found a well-marked increase (12 per cent.).

Of **osteomalacia** Neusser finds two types, with and without eosinophilia. The latter are usually advanced cases with inflammatory changes in the bones and marrow and myelocytes in the blood. The former include those cases in which castration has been followed by recovery.

Gout. Neusser first called attention to the high proportion of eosinophile cells in the blood of gouty subjects. Although his opinion was not supported by extensive reports, it will generally be found that in the gouty diathesis the eosinophiles reach Zappert's intermediate or high normal limits. Yet in testing this rule the writer soon found that this feature of the blood is of little value in the diagnosis of acute gout, meeting with cases in which the blood contained few oxyphile cells. In the irregular manifestations of the diathesis, eosinophilia appears to be more uniform but still subject to many variations.

Intestinal Parasites. Infection with various common intestinal parasites is accompanied by extreme eosinophilia, as first noted by Buckler in 1894.

In cases infected by *oxyuris*, Buckler found 19 per cent. of eosinophiles; by *ascaris*, 16 per cent.; *ankylostoma*, 72 per cent.; *tenia mediocanellata*, 34 per cent. In most of these cases eosinophile cells and Charcot-Leyden crystals are abundant in the feces.

Ankylostomiasis, however, is not always accompanied by eosinophilia or crystals in feces (Ehrlich). In the anemia from *bothriocephalus latus* Schumann found few eosinophiles in the blood. Trichinosis has lately been added to the list of diseases marked by extreme eosinophilia.

In cases of echinococcus cyst Dargein reports 12 per cent. of eosinophile cells.

Origin and Significance of Eosinophilia. The very diverse opinions that have prevailed regarding the origin of eosinophilia can only be finally reconciled by holding strictly to the view that eosinophile cells are derived solely from the eosinophile cells of the bone-marrow. That a local multiplication of these cells may occur under many circumstances is, however, strongly suggested by their abundance in certain foci, especially in the skin and mucous membranes. Thus, in the absence of general eosinophilia, rich deposits of eosinophile cells are often found in normal tissues, in cutaneous vesicles and pustules, in chronically inflamed mucous membranes, in nasal polyps and other tumors, and in leprous, tuberculous, and syphilitic lesions. Lately they have been found in very large numbers in bloody exudates in the pleura (Weiss[1]). Their abundance in the mucosa and exudate of the inflamed bronchi in asthma led Neusser, and, on parallel grounds, many others to actively maintain their local origin apart from the bone-marrow. In one case of pemphigus with marked local and general eosinophilia, Neusser[2] is reported to have found no increase of eosinophiles in the bone-marrow, but studies in this important field are yet entirely inadequate to figure in the discussion. In the marrow of cases of malaria showing eosinophilia the writer found an increased number of eosinophile cells, while in other cases without eosinophilia these cells in the marrow also were deficient. Dominici found an excess of eosinophiles in the marrow of a rabbit showing marked eosinophilia.

The exponents of the local origin of eosinophile cells have been unable to bring forward evidence to prove that these cells undergo mitotic division in the skin or that neutrophile granules are there transformed into eosinophile.

The writer believes that all the phenomena connected with general and local eosinophilia can best be explained by the same chemotactic principles that are known to control neutrophile cells. From the observations on eosinophile cells in gonorrheal pus, in cutaneous and serous exudates, and in the blood it appears that inflammatory products attract these cells at one stage and neutrophile cells at another and more acute stage. As purulent exudates of small extent may cause no general leucocytosis—*e. g.*, furunculosis—so, many cutaneous lesions with exudates composed largely of eosinophiles cause no general increase in the circulation, but when either reaches a certain grade the neutrophile or eosinophile cells are drawn from the marrow in sufficient numbers to cause a noticeable increase in the blood.

Ehrlich finds reason to believe that substances which attract eosinophile cells may be derived *from the destruction of epithelial cells*, as seen in the local eosinophilia about the ulcers of lupus after injection of tuberculin, *from mucin as in nasal polyps*, and *from toxins of parasites*. Michaelis has shown that when lactation is interrupted in the guinea-pig many eosinophile cells collect in the breasts ; but in the human breast after stagnation of milk Unger found many mast-cells.

Weiss has recently maintained that local eosinophilia tends to occur where there is extensive extravasation of blood, the derivatives of hemoglobin being absorbed by the polynuclear cells and deposited in the form of eosinophile granules. He bases this opinion on the observation of a case of septicemia with hemorrhagic pleural effusion. In the blood there were 40 per cent. of eosinophile cells, while in the sediment of the exudate 76 per cent. of these cells were found. He refers to two other somewhat similar cases reported by Harmser.

The most comprehensive view of the significance of eosinophilia is that of Neusser[1] and his pupils, who from extensive observations have found evidence that *the supply of eosinophile cells in the blood is controlled by the sympathetic nervous system, and that eosinophilia is the expression of sympathetic nervous irritation.* This irritation, they believe, may proceed from the generative organs, ovaries, uterus, or prostate, disorders of which, with their related neuroses, are usually accompanied by eosinophilia; from the skin, diseases of which have furnished some of the best examples of eosinophilia; from the intestines, from which the toxemia of parasites and that of gout, which Neusser regards as of intestinal autotoxic origin, give rise to marked eosinophilia, etc.

Following out this idea, Neusser has elaborated an extensive scheme of the altruistic relations of the viscera in disease, by which he is able to correlate nearly all the diseases showing eosinophilia. He finds a necessary sequence of events in a case which suffered from migraine in youth, pemphigus and asthma in old age, and died from cancer of the prostate with general metastasis in the bone marrow.

Although the recent tendency has been to deny any great importance in Neusser's conception of eosinophilia, it must be remembered that it is based on a well-known and far-reaching principle in pathology. While in practice a great many exceptions have been found to the rules which he formulated, it will yet be wise to keep them in mind in drawing conclusions from eosinophilia.

Value of Eosinophilia in Diagnosis. In general, the presence of a moderate or increased number of eosinophiles in the blood is often of value in the diagnosis between a condition in which these cells are known to persist as against those in which they are commonly absent. The writer has found situations of the sort most frequently in the diagnosis between *gouty* and *tuberculous* affections.

Other situations have arisen in the diagnosis between active malarial and typhoid fevers, scarlet fever and measles, trichinosis and rheumatism.

In prognosis the reappearance of these cells in suppurative processes is undoubtedly a favorable sign indicating the approach of defervescence.

Many clever deductions have been drawn by Neusser and his pupils from the behavior of eosinophile cells in the blood, but others have not been so successful in this field.

OCCURRENCE OF MAST-CELLS.

The knowledge of mast-cells dates chiefly from the studies of Westphal (1880) and of Unna, who found abundant collections of large mononuclear cells, with large, strongly basophile granules, in tumors, and in tuberculous, syphilitic, and other lesions of the skin. The occurrence of such cells in tissues has since been described by many writers, and it is now accepted that their appearance is favored by a considerable variety of chronic disturbances of nutrition in tissues, and that they are commonly associated with eosinophile cells. The majority of coarsely granular basophilic cells in tissues are not, however, identical with the mast-cells of the blood.

In the blood Canon failed to find them in nine of twenty-two healthy subjects, counting 500 to 1000 leucocytes, while in the other thirteen cases they yielded an average of 0.47 per cent. In a variety of skin diseases (twenty cases) they were slightly more numerous (0.58 per cent.), and, in general, were slightly increased when eosinophile cells were abundant. Sherrington found them distinctly increased in the blood of moribund cholera patients. Goldhorn finds that his method of staining is invariably successful in demonstrating mast-cells in normal blood. In cases of severe malaria the writer has seen them more abundant in the blood than in any condition except leukemia, while in the viscera of such cases large granular basophilic cells were unusually frequent.

Neusser examined a gonorrheal exudate composed exclusively of mast-cells, while Wolff reports 5 per cent. of mast-cells in a pleuritic exudate. Schmauch found marked increase of mast-cells in the blood of rabbits after injections of pyronin.

In myelogenous leukemia there is, in the majority of cases, mast-cell leucocytosis, these cells being here more abundant than in any other condition They often outnumber the eosinophile cells (Ehrlich), and must be considered the sole isolated pathognomonic sign of this disease. Ehrlich's statement (*Die Anaemie*, p. 123) that they are invariably present in considerable numbers in myelogenous leukemia may be capable of verification by recent methods, as Michaelis finds that the mast-cells of leukemia are much more soluble in water than those of normal blood; but with ordinary methods one may sometimes search in vain over thousands of cells in chronic cases.

Ehrlich believes that mast-cells are derived exclusively from the bone marrow, and respond to chemotactic influence from principles, however, which are very rarely present in the human organism.

OCCURRENCE OF MYELOCYTES (EHRLICH'S).

Although the eosinophile myelocytes and, to a less extent, Cornil's very large, pale neutrophile myelocytes are found almost exclusively in myelogenous leukemia, the smaller variety of mononuclear neutrophile cells have been shown to occur in the blood in a considerable variety of conditions.

From the many recent reports of the occurrence of a few myelocytes in the blood it appears that such cells may be swept from the marrow by several distinct causes:

1. ACCOMPANYING THE POLYNUCLEAR LEUCOCYTOSIS OF INFECTIOUS DISEASES, a considerable number of myelocytes may be found.

This fact first appeared in the studies of Turk, was emphasized through the discovery by Engel of unusually large numbers (12 per cent.) in unfavorable cases of diphtheria, and has since been extended by others to most severe, acute, and subacute infections.

Usually the percentage of these cells in polynuclear *leucocytosis* is much lower than in diphtheria, and not being associated with eosinophile cells or mast-cells, it can seldom raise a suspicion of leukemia. Nevertheless a difficulty arose with a case of acute leukemia, observed by Thomson and the writer, in which the first examination of the blood in a case resembling typhoid fever showed leucocytosis and 5 per cent. of myelocytes. A few days later there were 12 per cent. of myelocytes, and at the autopsy the lesions of leukemia were demonstrated. Eosinophiles and mast-cells were absent.

The presence of myelocytes in polynuclear leucocytosis is readily explained by the hyperplasia and hyperemia of the marrow.

2. IN PRIMARY AND SECONDARY ANEMIAS of severe or even of moderate grade, a few myelocytes have frequently been observed (Klein, Krebs, Loos, Hammerschlag, Capps). They are, perhaps, most frequently seen in pernicious anemia (Neusser), in the anemia of syphilis (Rille), with malignant tumors (Cabot), and in v. Jaksch's anemia. It has been supposed that tumors involving the marrow would yield myelocytes as well as eosinophiles in the blood, and Solley has recently described a case of sarcoma involving the bone marrow in which the blood showed 18 per cent. of myelocytes. They have been noted in considerable numbers in rachitis, osteomyelitis, and osteomalacia.

In all the above conditions the appearance of myelocytes must be referred to hyperplasia of red marrow, to mechanical dislodgement of marrow cells resulting from structural changes in the marrow or hydremic states of the blood plasma, and to chemotactic influences which may attract the ameboid myelocytes as well as the adult polynuclear cells.

3. AFTER SEVERE MECHANICAL DISTURBANCES OF THE CIRCULATION a few myelocytes may be found in the blood. This rule is illustrated by their discovery in the blood of uremia, asphyxia, acute mania, etc. (Neusser). The writer has seen a few myelocytes appear during antemortem leucocytosis.

The occurrence of myelocytes in leukemia will be considered with that disease.

LYMPHOCYTOSIS.

A relative or absolute increase of lymphocytes in the blood is of frequent occurrence and has at times important significance. In speaking of lymphocytosis, as of eosinophilia, since each of the cells concerned represents an independent series, conceptions will be more accurate if the actual numbers, as well as the relative proportions, are reported. In estimating lymphocytosis it is important to distinguish also between the large lymphocytes and large mononuclear leucocytes with faintly basophile protoplasm.

Physiological Variations. Physiological variations in the proportions of lymphocytes are observed at different periods of life. The first embryonal leucocytes are all mononuclear basophile cells (Saxer), but as other varieties of leucocytes make their appearance, the proportion of lymphocytes diminishes until, at birth, the healthy infant shows 50 to 66 per cent. of these cells. Throughout healthy infancy this proportion steadily diminishes until, at the tenth to the fourteenth years, the usual percentage is 27 to 30 per cent.

Pathological Lymphocytosis. Anything which interferes with the natural development of the infant retards the progress of this change in the proportion of lymphocytes, which is found to be relatively high in anemic or poorly developed children. Sometimes the development of lymphatic tissue reaches an excessive grade, and a condition known as "*constitutio lymphatica*" is established, marked by lymphocytosis, with simple hyperplasia of many lymph nodes and of the red marrow, by rachitis, and by many other developmental anomalies. Since Öhlmacher has demonstrated that most cases of *idiopathic epilepsy* are associated with the *constitutio lymphatica*, it is reasonable to suppose that lymphocytosis is a frequent feature of the blood in such cases. The lymphocytosis of *Basedow's disease* should probably be placed in this connection.

Rachitis is almost always attended with a well-marked increase in the number and proportion of lymphocytes in the blood (Rieder, Monti, Berggrun). This fact is plainly referable to the lymphoid hyperplasia and the hyperemia of the bone marrow in this disease.

In *some tumors* Reinbach has reported an extreme diminution of lymphocytes, only 0.6 per cent. being present in one case of *lymphosarcoma colli*. This result is referred by Ehrlich to closure of the lymph paths by sarcomatous growth. In many lymphomata, however, there is marked uniform lymphocytosis, and it would appear that these neoplasms when leaving the lymph paths free induce lymphocytosis. As the more malignant tumors obliterate lymph paths, some inference regarding prognosis may be derived from the presence or absence of lymphocytosis in these cases. Various other types of sarcoma are frequently associated with lymphocytosis and there are on record several cases of sarcoma associated with lymphatic leukemia. (See Leukemia.) In most *splenic tumors* there is relative or absolute lymphocytosis (Müller and Rieder, Weiss[2]). On the other hand, after splenectomy in animals, prolonged lymphocytosis is usually observed (Kurloff). In man, both mononuclear and polynuclear cells are usually much increased after this operation.

The lymphocytosis of infectious diseases is probably always associated with acute hyperplasia of the lymphatic structures.

In *typhoid fever* the hyperplasia is most marked in the abdominal nodes and spleen, but may become more general. There is in this disease a relative lymphocytosis, which after the first week usually increases until there is a high percentage of lymphocytes. The writer has found a uniform relation between the lymphocytes in the blood and the grade of lymphatic hyperplasia found at autopsy. In one case the examination of the blood led to a strong suspicion of lymphatic leukemia, and at autopsy the mesenteric glands were of unusually large size, and the edges of the partly necrotic intestinal ulcers rose 1.5 cm. above the mucosa.

In *the infectious diseases of children* a well-marked lymphocytosis, together with increase of neutrophile cells, is so common that it should occasion no surprise, and points merely to a special involvement of the lymphoid tissues.

In *diphtheria* a close relation between lymphocytosis and lymphatic hyperplasia was noted in some of the writer's cases. In others, however, the lymphocytes, though increased, were not in relatively high proportion, while the cervical lymph nodes were much enlarged.

In *bronchopneumonia* Cabot reports a case with 94,600 leucocytes, 69 per cent. of which were lymphocytes

In *whooping-cough* during the convulsive period Meunier found the neutrophile cells doubled, but the lymphocytes quadrupled in number.

During the course of *measles*, at the close of *scarlet fever* or *small-pox*, and during prolonged lysis in *pneumonia* (Klein), a relatively high proportion or increased number of lymphocytes is usually observed.

In the less acute *diarrheas of infancy* the lymphocytes are usually slightly, and sometimes very much, increased over the high proportions normally existing at that age, as a result of the hyperplasia and irritation of the intestinal lymphoid structures (Weiss[2]). *Digestion leucocytosis* is also for the same reason largely a lymphocytosis (Rieder). In the initial or persistent hypoleucocytosis sometimes observed in infectious diseases (pneumonia, diphtheria) the remaining cells are chiefly lymphocytes.

Of *chronic diseases* affecting the lymphatic tissues both tuberculosis and syphilis are frequent causes of lymphocytosis.

In various forms of *pulmonary and visceral tuberculosis* the leucocytes are usually normal or diminished in number and the majority of those remaining are lymphocytes, a relation which is, of course, altered when from any cause polynuclear leucocytosis is established. The condition of the blood is here in accord with the histological character of the visceral lesions in which the infiltration with "round cells" is a prominent feature.

Lymphatic anemia is a term applied by Neusser to a rather characteristic group of cases in which there is chlorotic anemia, lymphocytosis, and, usually, evidence of tuberculosis. Rieder could not agree with Neusser that most cases of chlorotic anemia with increase of lymphocytes are associated with tuberculosis and are of less favorable prognosis. The writer finds that the chlorotic anemia of tuberculosis, as a rule, shows *absence of eosinophile cells* as well as increase of lymphocytes.

Injections of tuberculin are usually followed by fever and lymphocytosis, which may be referred to irritation of inflamed tuberculous lymph nodes (Ehrlich).

In *congenital and in secondary acquired syphilis*, it has been shown by Bieganski, Rille, Anc, and others, that there is uniform and considerable lymphocytosis, which must here again be referred to the involvement of lymph nodes.

The presence of an increased proportion of lymphocytes in some cases of *scurvy* and *hemophilia*, has been observed by Neusser, and the same feature is recognized by Ehrlich in many *severe anemias*. In *anemia infantum pseudoleukemica* the very numerous leucocytes are principally large mononuclear cells. In *pernicious anemia* a considerable leucocytosis may consist chiefly of lymphocytes.

Experimental Lymphocytosis. Experimental lymphocytosis has been produced by injections of tuberculin and of extract of carcinomatous tumors by Grawitz, who refers this effect to the lymphogogic action of these agents. Waldstein reports lymphocytosis in rabbits from pilocarpine, which the writer finds to induce in rabbits only a relative increase of lymphocytes by diminution of polynuclear cells.

In the *origin of lymphocytosis* Ehrlich finds that mechanical rather than chemotactic influences are chiefly or wholly concerned; but reasons have already been given for doubting the entire truth of this conclusion, since the lymphocytes possess ameboid properties and may be sensible to chemotactic influences.

The histology of tuberculous and syphilitic lesions, especially the "lymphoid tubercle" and chancre, indicate that there are some conditions which gather large numbers of these cells from the blood and lymph nodes. Janowsky's experiments in which large collections of lymphoid cells were shown to gather at the site of injection of turpentine, etc., point to the same conclusion. In all of these situations, however, the lymphocytes, like eosinophile cells, seem to follow entirely different rules from those governing neutrophile cells —a fact which emphasizes the importance of Kanthack's classification of lymphocytes and eosinophiles as celomic, the neutrophile as hemic cells.

Relation of Lymphocytosis and Eosinophilia. It is of interest to note that a peculiar relation seems to exist between the appearance in blood and tissues of lymphocytes and eosinophile cells. Neither are apt to appear when neutrophile cells are prevailing in blood or exudate, but either may be found in the less acute exudative processes. The sequence of lymphocytosis followed by eosinophilia is especially clear after the injection of tuberculin (Grawitz) and after splenectomy in animals (Kurloff). Many other less patent examples of this relation may be noted by comparing the conditions showing lymphocytosis or eosinophilia, as above detailed.

Large Mononuclear Leucocytes in Lymphocytosis. In the majority of cases reported as showing lymphocytosis no distinction has been made between lymphocytes and large mononuclear leucocytes. Yet there is considerable evidence to show, as Ehrlich believes, that these cells represent separate series, and in some conditions it is the large mononuclear leucocytes and not the lymphocytes which are increased in number. This fact has been noted in *v. Jaksch's anemia*, especially by Hock and Schlesinger; in rachitis by Rieder; in syphilis by Rille; and after smallpox and scarlet

fever by Felsenthal.[2] It is sometimes distinctly seen after splenectomy In the cases of acute lymphemia, described by Frankel and others, the majority of cells are of large size. The significance of an increase of large mononuclear cells apart from lymphocytes cannot at present be stated.

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CHAPTER V.

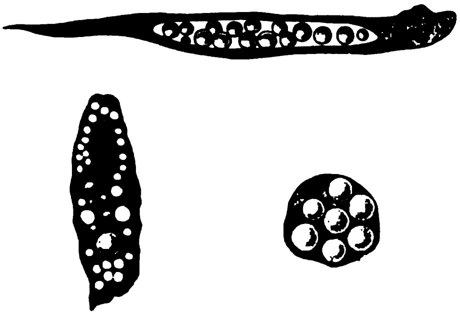
DEVELOPMENT OF BLOOD CELLS.

WHILE the mode of origin of the first blood cells of the embryo has been fully determined, the alterations in the process which supervene during late fetal and adult life still remain an obscure and difficult problem. It becomes necessary, therefore, to consider separately the formation of blood cells at different periods of intrauterine and extrauterine life.

ERYTHROCYTES.

Formation of Red Cells in the Embryo. In early embryonal life the formation of red cells in isolated groups of mesodermal cells in the "vascular area" of the chick was first noted by Pander, who named these foci "blood islands." The process was later studied more closely by His, Remak, Kolliker,[1] Wissosky, Klein, Stricker, and others. From their researches it appears that the first blood cells of vertebrates are formed by the appearance of Hb in some of the cells of the mesodermal cords which go to form the first capillaries. Upon the formation of the vessel these cells lie free in the lumen as nucleated red blood cells. Both the vessel wall and the primitive erythrocytes are thus derived from the same group of cells.

FIG. 31.



Theory of endoglobular formation of red cells. (Schäfer.)

Theory of Endoglobular Formation of Red Cells. In many mammals all the early red cells are nucleated, but in some (rat, guinea-pig) many non-nucleated red cells are present at an early stage, although in the human embryo they appear certainly only after the fourth week and form only 25 per cent. of the red cells at the fourth month. The formation of the first non-nucleated red cells has been studied by Schäfer, Ranvier, Hayem, and others, who describe in the rat and guinea-pig the appearance in certain large connective tissue cells (angioblasts, hematoblasts, *cellules vasoformatives*) of disks and globules of hemoglobin, which, by subdivision of the body of the cell, become non-nucleated red blood cells. These red cells soon come to lie free in a central cavity within the cell, which is itself transformed into a young capillary. According to this theory, the *red disks are not cells, but fragments of cells of endoglobular origin, while nucleated red cells have a different significance, being formed by mitotic division of other nucleated red cells.* With the development of the lymphatic system and liver, the intracellular formation of red cells is said to cease, and few or no traces of it have been described in most mammals at birth. Kuborn and Malassez, however, describe the formation of red cells in the late embryonal liver and marrow through gemmation of the giant cells of these organs.

The theory of intracellular formation of blood disks has not been supported by more recent investigations. Spuler and Saxer find no traces of such a process, regarding the vasoformative cells of Ranvier as endothelial cells which have become separated from the delicate young vessels, and have carried with them some of the red cells of the vessel. In the adult marrow such cells have not been fully identified. Recently, however, Francois and Poljakoff have attempted to substantiate the theory of endogenous red-cell formation. Poljakoff placed cover-glasses separated by a capillary space beneath the skin of guinea-pigs for twelve to twenty days. The glasses and inclosed cells were immersed in osmic acid and stained by carmine. In such specimens he describes the formation of red cells and elaboration of Hb by the linin substance of the nuclei of leucocytes and fibroblasts. He says nothing of the difficulty of recognizing Hb under such conditions, but is certain that the acidophile staining masses are not englobed fragments of red cells.

In *later embryonal* periods the process of development of red cells is very similar to that in extra-uterine life.

Mode of Origin of Red Cells in Late Embryonal and Extrauterine Life. That the nucleated red cells of the marrow multiply by indirect division of other nucleated red cells was first stated by Bizzozero,[1] and has been accepted by most histologists. Bizzozero, however, claimed that the large nucleated red cell of the adult marrow is the most primitive cell in the body capable of producing erythrocytes, believing that the intermediate stages between the embryonal wandering cell and the colored erythroblast have been lost in later embryonal life; but it has since been shown to the satisfaction of most authorities, that the series of mitoses leading to the production of red disks begins in colorless cells antecedent to the large nucleated red cells, and which are only very slightly different from the original mesoblastic cell of the embryo. It thus appears that the formation of nucleated red cells in the adult is practically the same as in the embryo and that at all periods of life the red cell is the product of several series of mitoses of a colorless mesoblastic cell. The difficulty of tracing this series from the large nucleated red cell to the colorless mesoblastic "mother cell" in the marrow has given rise to the diverse opinions now held regarding the ultimate development of red corpuscles.

As to the exact *morphology of the original erythroblasts of adult marrow,* opinions are more or less at variance, but it is agreed by Neumann,[2] Rindfleisch, Obrastzow,[1] Malassez, Howell, Löwit,[1] Müller, and many others, that it is a large mononuclear cell, larger than the ordinary nucleated red cell, with pale nucleus and without hemoglobin, and it is usually described as lacking a nucleolus. From these "embryonal" cells are derived, by mitotic division, two or more series of cells which gradually approach the type of the nucleated red cell, which is rich in hemoglobin and whose nucleus is compact and pyknomorphous.

Among dissenting opinions may be mentioned the conclusions of Foa, according to whom the red cells are derived from the giant cells of the marrow, spleen, and liver, and the theory of Hayem,[2] partially accepted by Afanissiew and Pouchet, that the red disks are derived from the blood plates.

Regarding important details in the *origin and relation of the formative erythroblasts,* opinions are also widely at variance.

Löwit describes in the marrow, spleen, and lymph nodes, two separate series of colorless cells which produce, one, leucocytes, the other, red cells. The cells of these two series differ in the structure of the nucleus, in the fact that one type (leucoblasts) exhibits ameboid and phagocytic activity, and in their method of division, the erythroblasts multiplying by indirect division, the leucoblasts by a modified form of karyokinesis described as *divisio indirecta per granula.* According to Löwit the red cells are derived from the erythroblasts after these have reached the circulation from the lymphoid tissues.

Bizzozero[2] describes the primitive erythroblast as separate from the leucoblast, both in morphology and especially in its relation to the bloodvessels of the marrow. While numerous nucleated red cells in mitotic division are found, according to Bizzozero, in the masses of cells lying within the capillaries of the marrow of birds, intervascular tissue is entirely free from such cells. The capillary network of the marrow is, therefore, an endovascular blood gland. This observation has been verified by Torre, Salvioli, and Denys, for nucleated hemoglobin-holding cells, but other authors trace the erythroblasts back to colorless cells, which are not invariably intravascular. In man, however, while the erythroblasts lie closer to the lumen of the capillary than do the leucoblasts, the walls of the sinuses are incomplete and the islands of nucleated red cells are found in the midst of masses of leucoblasts (Muir).

H. F. Müller describes a common form of mother cell for both red and white blood cells. By their indirect division are produced (1) cells which resemble the original, (2) mononuclear leucocytes, and (3) nucleated cells which develop hemoglobin and by the gradual disappearance of their nuclei become the non-nucleated red blood disks.

Denys believes, with Löwit, that there are two separate developmental series of leucoblasts and erythroblasts, each of which multiplies by mitosis, and is originally colorless. Both are ameboid, but the erythroblasts are very slightly so. Denys, like Bizzozero, finds that the leucoblasts lie in the cords outside the vessels of the marrow, while the erythroblasts are found within the vessels.

Hayem believes the red blood disks to be derived from the blood plates of Bizzozero, which he therefore calls "*hematoblasts.*" He claims to have seen all transition forms between blood plates and red blood cells, and finds that when blood formation is unusually active the blood plates and the transition forms are more abundant. In regard to many details of the origin of blood plates and their transformation into red cells, Hayem was unable to reach satisfactory conclusions.

Naegeli traces the progenitors of the erythrocyte back to large and small non-granular cells free from Hb, with reticulated oval nuclei, but without nucleoli. Except for the absence of nucleoli this description applies exactly to large and small lymphocytes.

Pappenheim argues strongly in favor of the existence in both embryonal and adult marrow of a common cell of origin of both red cells and leucocytes. This cell he describes as indistinguishable morphologically from the large or small lymphocytes. His conclusions are based on theoretical considerations and on a study of embryonal and adult marrow submitted to a great variety of staining methods. He claims to find all transitional stages between lymphocytes and megalocytes on the one hand, and granular leucocytes on the other. From this primitive lymphocyte are developed the differentiated lymphocyte of the circulation, which is incapable of further change and always remains a lymphocyte, while from other types of metaplasia are developed hemoglobiniferous cells and granular leucocytes.

It seems at present impossible to decide how much credence must be given to claims that it is possible to trace hemoglobiniferous cells in the adult back to cells which are entirely free from Hb, and which resemble lymphocytes. The writer hesitates to accept these claims, because simple but highly differential staining (hematoxylon and watery eosin) has, in his hands, always cut off the hemoglobiniferous cells of the marrow quite distinctly from those which are free from Hb.

To summarize the work in this field, it may be said that we do not know certainly whether any common cell of origin of red and white blood corpuscles exists in the late embryo or adult, or whether these corpuscles are derived from completely separate series. The late contributions favor the existence of a common mother cell for both groups, persisting at least into late embryonal life.

The Transformation of Nucleated Red Cells into Blood Disks. To that minority of observers who believe that the red blood disks are derived from other sources than the nucleated red cells, the task of explaining the disappearance of all trace of the nucleus presents no difficulties. Among the majority of writers who accept the origin from nucleated cells, the theories regarding the disappearance of the nucleus are various, but none has been fully demonstrated.

Kolliker,[1] Bizzozero,[3] Neumann,[1] Löwit, Foa, and many recent observers believe that the nucleus gradually disappears in the cell. The difficulty in accepting this opinion lies in the fact that while red cells with rather small nuclei are abundant in red marrow, the *final stages* of the disappearance of the nucleus are traced only with difficulty; yet Schmidt, Spuler, Israel and Pappenheim, and Masslow, claim to have found these final stages in abundance. Ehrlich believes that the nucleus of the normoblast is extruded, while that of the megaloblast fades within the cell.

Rindfleisch first, and later Howell, described fully the shrinkage and *final extrusion of the compact nuclei of red cells* in man, mammals, and amphibia. Van der Stricht, Kostianecki,[1] Saxer, Albrecht, Disse, Muir, and others, find that after considerable shrinkage the nucleus may become compact and homogeneous or may be fragmented, but it is eventually extruded, and either breaks up in the plasma or is englobed by leucocytes, giant cells, or endothelia. The significance of this somewhat remarkable phenomenon has not been fully demonstrated. The opponents of this theory claim that free nuclei are seen in sufficient numbers to account for the production of many red cells, *only in smears* of the marrow, but scantily *in sections*. The writer finds this objection valid, unless the destruction of extruded nuclei is more rapid than we have any good reason to believe.

As an antithesis to the "extrusion" theory, Malassez, Fellner, and Duval believe that the nucleated red cell extrudes a portion of its substance in the form of a red disk entirely free from nuclear material, but the histology of the marrow offers insufficient ground for the belief in such a process.

Engel[1] holds that megaloblasts produce megalocytes by gemmation, leaving normoblasts, from which the nuclei are extruded. Mondino and Sala find evidence that the nuclear material finally diffuses along the periphery of the cell, where it undergoes a chemical change which causes it to lose its affinity for basic dyes. Obrastzow[2] also believes that the nucleus persists in diffuse form in the disk, and that it may reappear as a result of postmortem processes. Finally, Bottcher and Brandt claim that the nucleus persists and may be demonstrated in the normal red cells by certain staining methods, while Wooldridge and Botazzi and Capelli, by chemical analysis, are always able to demonstrate nuclear material in the red cells.

While the weight of opinion and evidence seems to favor the gradual fading of the nucleus within the cell, and while it can hardly be denied that extrusion frequently occurs, there is abundant evidence to show that the red cell is not entirely devoid of nuclear material. The chemical analysis of the stroma of these cells decidedly favors the presence of nuclear elements (Lilienfeld, Wooldridge); the appearance of degenerating cells strongly suggests a nuclear remnant, and the employment of recent stains—neutral red, polychrome blue—frequently yields the specific reaction of chromatin in the centres of slightly altered red cells The recent studies of Maximow indicate that while the bulk of the nucleus is extruded, a portion remains in finely granular form and imparts a basic staining quality to the centres, especially of young red cells.

Seats of Formation of Red Cells. (*a*) *In the early embryo* the production of red cells follows the development of capillary bloodvessels in many tissues. Very soon the rudimentary liver and lymph nodes make their appearance and in them the function of red-cell formation is gradually concentrated.

(*b*) *In the fully developed embryo*, the majority of investigators locate the chief formation of red cells in the liver, to a less extent in the spleen, while the bone-marrow, which is at first less prominent in this process, gradually absorbs the function, and in the human fetus at birth represents its chief seat.

In most infants at birth, however, the liver, and less constantly the spleen, are found to contain many nucleated red cells, while in some cases the proportion of these cells in the liver is very large and numerous small collections of them may be found scattered along the portal capillaries.

(*c*) *In Extrauterine Life.* The fact that the red marrow is probably the exclusive depot of formation of red cells in the adult was discovered almost simultaneously in 1868 by Neumann[3] and by Bizzozero.[1]

This original claim has been uniformly substantiated by those later observers who recognize as erythroblasts only those cells which contain a trace of Hb. Others, as Löwit and Müller, Gibson, Foa, and Saxer, who trace the red cells back to colorless cells, regard all lymphoid tissues as probable sources of erythroblasts.

In pathological conditions it has been shown that the spleen (Bizzozero,[3] Neumann,[1] Howell) in the human adult may resume its embryonal function of red-cell formation, as indicated by the presence of many nucleated red cells. Under similar conditions it has been shown that the limits of red marrow, normally confined to the flat bones, ribs, vertebræ, and upper and lower thirds of the long bones, may be extended throughout nearly the entire cavities of all bones except those of the feet (Neumann, Litten and Orth, Bizzozero and Salvioli).

In infancy, and up to the sixteenth year, the cavities of all bones contain almost entirely red marrow, but there are considerable variations in the proportions of lymphoid and fat tissue in the shafts of the long bones. It is probable that in most healthy *infants* the formation of red cells is already limited to the lymphoid marrow, but considerable numbers of nucleated red cells, often grouped in islands, are rather frequently to be found in the spleen and liver of apparently healthy asphyxiated infants. In the acute and chronic anemias of infancy and childhood, the facility with which the spleen and liver resume their former *rôle* of red-cell production partly explains the special involvement of these organs in such diseases.

LEUCOCYTES.

Development of Leucocytes. The earliest indications of the formation of leucocytes are seen in the presence of primary wandering cells, of mesodermal origin, which are found principally in the loose connective tissues of the early embryo. The specific quality of these wandering cells must stand as one of the fundamental facts connected with the development of blood cells. Though of mesodermal origin they are from the first quite distinct in morphology and apparently in function from the capillary endothelium and fixed connective tissue cells. The primary wandering cells are of large size—8μ to 9μ (Saxer)—with a single large nucleus containing one or more nucleoli and with a moderate quantity of finely granular, slightly acidophile protoplasm. Their development has been traced by H. E. Ziegler to masses of mesodermal cells surrounding the cords from which the capillaries are formed. It thus appears that the parent leucocytes lie originally *outside the vessels*, into which they make their way by virtue of ameboid powers.

The identity of the primary leucocytes with the primary wandering cells has been generally accepted, but Schmidt, Bonnet, and others, claim that leucocytes are derived from the capillary endothelium. The weight of evidence, however, is much against this view, which inserts the highly specialized endothelial cell as a transition stage between the primary mesodermal cell and the leucocytes. An isolated position also is assumed by v. Davidoff, Maurer, Beard, and others, who believe the leucocytes to be direct derivatives of the *epithelial cells*, principally of the intestine. This view is based upon the appearance of lymphocytes in and between the epithelial cells lining many mucous surfaces, which has been repeatedly and fully explained as an infiltration by leucocytes, not a production of new lymphocytes from these cells (Saxer).

Beard very positively maintains that in *Raja* the first leucocytes are found in the epithelial masses of the thymus at a stage of development (17 mm.) when no leucocytes are seen in any other tissue. He describes the stages of transformation of these epithelial cells into leucocytes. This is Kolliker's opinion, first expressed in 1846.

The white blood cells of vertebrates make their appearance in the circulation only after the development of an extensive vascular system and long after the red-cell formation has been fully established. In the lower vertebrates their absence has been noted nine weeks (Ziegler[1]) after the appearance of red cells, but Hayem found them in frogs' blood thirty-four days after the appearance of red cells. The time of their appearance in the blood of the human embryo has not been accurately fixed. Gulland finds leucocytes in lymph vessels in the human embryo of one and one-half inches in length. In form the earliest leucocytes of the embryonal circulation are the small and medium-sized basophilic lymphocytes.

Process of Development of Leucocytes from Primary Wandering Cells. Most observers find that the primary wandering cells produce by mitotic division one or more generations of colorless cells which gradually approach in morphology the early basophilic leucocytes of the circulation. Most of the obscurity which still surrounds the early development of red and white blood cells is connected with this stage of the process, and the numberless conflicting opinions have resulted from the different interpretations placed upon the intermediate stages between primary wandering cells and circulating blood cells.

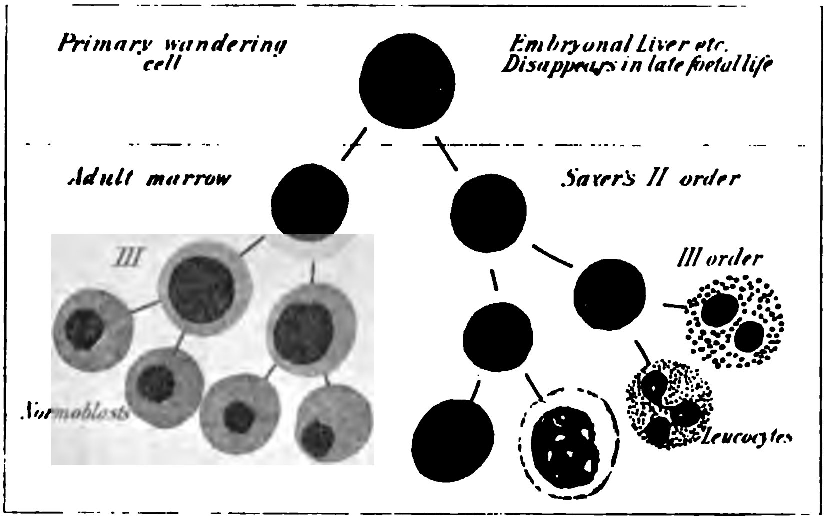
Denys, Löwit,[1] Ziegler,[2] v. d. Stricht, and others, claim that red cells and leucocytes develop from separate series of cells which have become differentiated from the primary mesodermal cells with the first appearance of blood and bloodvessels.

Kostianecki,[2] Müller, Schmidt, Saxer, Pappenheim, and others, believe that the primary wandering cell persists in the blood-forming organs as the parent of both red and white cells.

It is not impossible to partially reconcile these opposing views. While the exhaustive studies of Saxer seem to leave no room to doubt the common origin of *embryonal* red and white cells, it by no means follows that the blood cells of the *adult* may be traced to a common origin. Several generations (three to four, Saxer), each multiplying by indirect division, are required before the primary wandering cell is transformed into red cells or leucocytes. It is quite possible that the first members of the series, including the primary mesodermal cells, long persist in the embryo, but *disappear in the adult*, in whom, therefore, no common cell of origin of erythrocytes and leucocytes can be demonstrated.

A review of the principal minute studies in this field seems to the writer to warrant the above general conclusion in regard to the relation of red and white blood cells. That all blood cells of the embryo can be traced to a common origin seems to be fully proven. Although the exhaustive studies of Denys, v. d. Stricht, and Löwit were largely upon embryonal tissues, the morphological criteria on which they separate primary leucoblasts from erythroblasts do not appear sufficiently distinct to convince other observers, while the demonstration by Saxer and others of earlier mitotic cells in the same embryonal tissues seems to show that the common cell of origin persists in the fetal liver. Müller's studies of leukemic blood, in which he seems to have traced leucocytes and red cells to a common origin in the adult, indicate that this pathological condition involves a reversion to the embryonal type of blood formation, but does not prove that a common mother cell of both red and white blood corpuscles persists in the normal adult marrow. (Fig. 32.)

FIG. 32.



There is a constantly increasing number of studies which have led to the conclusion that there is a developmental relation in the adult between all colorless blood cells, and that the indifferent lymphocyte is their common progenitor. The views of Naegeli and Pappenheim have already been considered (p. 177).

Wolff describes the indifferent lymphocyte as larger than the small lymphocyte of the blood, with round or slightly indented nucleus and non-granular cytoplasm which is less basophile than the nucleus, while in the differentiated lymphocyte the cytoplasm is more basophile than the nucleus. Dominici thinks the primitive lymphocyte is indistinguishable, morphologically, from the small lymphocyte of the blood. From an extensive study of the inflamed omentum he concludes that polynuclear neutrophile cells develop both from myelocytes, and from lymphocytes without passing through the myelocytic stage. The myelocytes are developed from the smaller homogeneous basophile cells of the marrow.

Cellular Division in the Development of Leucocytes. Since the full demonstration by Flemming of numerous mitotic figures in the cells of the lymph follicles, karyokinesis has been accepted as the chief method, and many claim it to be the only method, of multiplication of leucocytes.

This view has been actively combated by Löwit, who finds that while erythroblasts multiply by mitosis, leucoblasts follow a modified form of the process which he calls *divisio indirecta per granula*. This author has very ably and successfully defended the importance of direct division in mammals and lower vertebrates, and subsequent writers have admitted that this method is of "very widespread" (Saxer) occurrence among leucocytes. Direct division appears to be of special importance in the development of the giant cells of the embryonal liver and adult marrow, which play an important but somewhat obscure part in blood formation. *Indirect fragmentation* is a term applied to a somewhat peculiar modification of the usual process of nuclear division described by many authors, especially by Arnold.[1]

While the formation of leucocytes occurs principally in the blood-forming organs, many have found in the circulating blood, and especially in the lymph, evidences of active multiplication by mitosis and more frequently by amitosis (Spronk and Prins, Löwit,[1] Wertheim).

Sites of Origin of Embryonal Leucocytes. Before the leucocytes begin to appear in the circulation mitotic figures are abundantly seen in the primary wandering cells in various situations. These are gathered in groups, first in the loose connective tissues of various regions, where lymph nodes subsequently develop; but the chief seat of the production of leucocytes, as of red cells, is found in the embryonal liver. In both situations the wandering cells are found in the lymph and blood capillaries, in the interstices of the connective tissues, and between the liver cells. In later embryonal life the formation of leucocytes is gradually transferred from the liver to the lymphoid and adenoid tissues, as indicated by the development of the lymph nodes, spleen, marrow, and thymus. While the majority of observers believe that these tissues are at all times developed by the proliferation of extravascular wandering cells and leucoblasts, Stohr and Gulland believe that these cells multiply principally *within the vessels* and subsequently wander out at certain localities, where they collect to form lymph nodes.

Formation of Leucocytes in Adult Life. Under normal conditions the reproduction of leucocytes in the adult is limited to the lymphoid structures, including the lymph nodes, spleen, marrow, and hemolymph nodes.

Hemolymph Nodes. Warthin has recently drawn attention to the considerable importance of the hemolymph nodes in the development and destruction of red and white blood cells. The existence of these nodes has long been known, but until recently they have received only desultory notice from pathologists.

Warthin divides the nodes into two types resembling the spleen and the marrow. Transitional forms occur, and the types appear to undergo cyclical changes with occasional reversion to the simple lymph node. The splenic type is abundantly present in all animals in the retroperitoneal and thoracic regions, while the myeloid type is limited to the retroperitoneal region. The structure of the splenic type is that of a small lymph node with blood sinuses, but without distinct Malpighian bodies. Drummond finds smooth muscle in nearly all cases and sometimes lymphoid sheaths about small arteries. He states that the characteristic feature is the large size of the sinuses and the presence of macrophages holding pigment and red cells. Warthin finds that in physiological states the hemolymph nodes are scanty and seem to play little part in blood formation, but in pathological conditions the nodes increase in size and number, new ones forming in lymph nodes and fat tissue, and they exhibit pronounced signs of red-cell destruction, although never forming red cells. In diseases of the blood they undergo changes homologous with those of the spleen and marrow. In pernicious anemia he finds the hemolymph nodes increased in size and number and exhibiting very marked signs of destruction of red cells, and points out that the enlargement of the lymph nodes reported in this disease by many observers is probably referable to this type of lesion.

Appearance of Different Varieties of Leucocytes. In the later months of fetal life the blood begins to show the presence of other varieties of leucocytes besides the lymphocytes. The minute morphology of these early cells and the exact time of their appearance is not known, but it is certain that all varieties seen in the adult are present at birth, and there is much evidence to show that long before this period the development of several distinct types of leucocytes is well established and their formation at least partially limited to certain viscera.

Sabrazés and Muratet examined the blood of a human fetus of the eleventh week of gestation. The proportion of red to white cells was 1 : 400. There were twelve times as many non-nucleated as nucleated red cells, and almost as many megaloblasts. The normoblasts were polychromatic, the megaloblasts and non-nucleated cells orthochromatic. The leucocytes were mostly medium-sized and large mononuclear cells. Polynuclear neutrophile cells were very scanty, and no eosinophile cells could be found.

The Basophile Leucocytes. These cells are the direct descendants of the first embryonal leucocytes, and are undoubtedly produced, in the adult as in the embryo, by the mitotic division of cells lying in the proliferation zones of lymph follicles, and diffusely in all adenoid tissues. Virchow regarded the small basophile cells (*lymphocytes*) as originating in the lymph nodes and the large basophile cells (*splenocytes*) as derivatives of the spleen; but this distinction has not been supported. It seems more probable, though by no means certain, that most or all of the larger basophilic leucocytes of normal blood are developed from lymphocytes. Kanthack and Hardy endeavored to subdivide this class into basophile and hyaline cells, the former being "tissue cells" and rarely appearing in the blood, the latter identical with the ordinary small and large lymphocytes. Kanthack's coarsely granular basophile cell is the "*mast-cell*," which has no connection with the lymphocytes; but his finely granular basophile cell has not been recognized as distinct from the hyaline cells whose protoplasm is not strictly hyaline but reticulated, and which undoubtedly originates in the lymphoid organs.

The *mast-cells*, which contain large, strongly basophilic granules, are classed by Kanthack as celomic (tissue) cells. They are quite different in appearance from the coarsely granular basophile cells of chronically inflamed tissues, and their exact origin has not been explained. (See "Mast-cells," Section on Leukemia.)

The Neutrophile Leucocytes. The time of appearance of these cells in the human embryo is not known. Saxer finds in the sheep's embryo of 4.5 cm., in connective tissues and scantily in the lymph, polynuclear leucocytes identical in appearance with those of the adult animal.

If the polynuclear leucocytes are developed from lymphocytes, the seat of their production in the infant at birth is, of course, coextensive with that of lymphocytes; but if polynuclear neutrophile cells are derived as a separate series from the neutrophile myelocytes, their formation is limited, at birth and thereafter, to the red marrow. Whichever view may be correct, it is certain that the neutrophile myelocytes are largely concerned in the formation of these cells, and that the marrow is the chief seat of their production.

The opinion widely held that neutrophile leucocytes are developmental forms of the lymphocyte of the blood still lacks any definite support. That they are certainly produced by mitotic division of neutrophile myelocytes has, however, been placed beyond question by the constant appearance of increased numbers of mitotic myelocytes in leukemia and leucocytosis. The larger size, distinctly vesicular nucleus, basophilic protoplasm, and invariable absence of neutrophile granules in the most typical examples of the so-called "transitional leucocytes" cannot be reconciled with the view that these cells are ever transformed into neutrophile leucocytes. Some have referred to myelocytes deficient in neutrophile granules as transitional forms, but in leukemia where such cells are common, a deficiency of neutrophile granules is very frequent in both myelocytes and polynuclear leucocytes. Again, if basophile leucocytes form neutrophile cells it is difficult to see why numerous transition forms are not found in lymphatic leukemia where basophile cells are excessively numerous, but neutrophile are abnormally scarce. Löwit here resorts to the suggestion that the further development of these cells is inhibited by changes in the plasma. Yet in myelogenous leukemia no such changes in the plasma prevent the transformation of myelocytes into polynuclear leucocytes which here often outnumber the myelocytes.

Out of the constantly accumulating evidence which goes to establish the specific qualities of different leucocytes may be mentioned the studies of Zenoni, who found no change in the proportion of neutrophile cells during very marked variations, experimentally induced, of the basophile.

In health, Dominici finds that the polynuclear cells are developed exclusively in the marrow and from myelocytes. In the intestinal lymphoid tissue and omentum of typhoid infections he finds lymphocytes changing into myelocytes, and in the lymph nodes on the third day of vaccinia in the rabbit he finds lymphocytes changing into polynuclear cells without the intermediate stage of the myelocyte.

Bezancon in pneumonia, and Frankel and Japha in scarlet fever, and Dominici, describe an active formation of polynuclear cells in the spleen. The writer has seen in the spleen of pneumonia a great abundance of polynuclear leucocytes in the pulp cords of the organ, but only in cases of general pneumococcus septicemia, in which the condition may be regarded either as a transformation of the original mononuclear cells of the cords into polynuclear, or as an infiltration from the blood stream by polynuclear leucocytes. Such conditions do not seem to present favorable opportunities for the demonstration that polynuclear neutrophile cells may develop from the mononuclear basophile cells of the spleen. More suggestive of such a development is a specimen of the spleen from a case of puerperal streptococcus septicemia, recently encountered by the writer, in which this organ is the seat of multiple miliary abscesses, in some of which all the cells are polynuclear, in others, nearly all are mononuclear, while numerous transitional stages are to be found.

The Eosinophile Leucocytes. These cells very early become differentiated from the primary leucocytes, having been found in the chick's blood on the fifth day of incubation (Engel[2]) and in the human thymus and lymph nodes before the appearance of bone marrow (Schaffer, Gulland). Though it was early found that they are specially abundant in the marrow, their very wide distribution in the tissues shows that the conditions essential to their development are not limited to any single organ or tissue. The discovery of mitotic division of eosinophile leucocytes, first reported by Müller and subsequently verified by many others, would seem sufficient proof that these cells constitute a distinct self-perpetuating series. Nevertheless, many current writers either tacitly accept or actively argue that the eosinophiles are derived from the neutrophile granules of polynuclear leucocytes. Demonstration of the truth of this view is at present entirely lacking. Only Gulland attempts to describe transitional stages between neutrophile and eosinophile granules in embryonal blood cells, but his claims have not only not been verified, but are distinctly contradicted by the results of the vast majority of observers, who have failed to find any such transition forms in the adult. On the other hand, there is abundant physiological, morphological, and microchemical evidence to show that eosinophile leucocytes are not derived from neutrophile.

Physiologically, the eosinophile cells are celomic tissue cells, finding their natural habitat in the tissues and not in the blood, whereas the neutrophile leucocytes are chiefly hemic cells occurring almost exclusively in the blood. Moreover, since these cells certainly multiply by mitosis, it is unnecessary and contrary to analogy to suppose that any other method of development or formation exists. The *morphological evidence* includes the fact that transitional granules have not been demonstrated; that the nuclei of the two cells have rather distinctive characters, and that the granules are integral parts of the cytoreticulum (Heidenhain, Gulland), and not, as has been supposed, excretory products in the cell. The *microchemical evidence* shows that the eosinophile granules yield Lilienfeld's and Monti's reaction for phosphorus (Sherrington), may contain iron (Barker, Arnold), and give the vanillin and aldehyde reactions of Weiss, none of which characters have been demonstrated in neutrophile granules.

THE BLOOD PLATES.

The blood plates were first described by Donne, in 1842, who found them in blood upon the addition of water and regarded them as particles of globulin derived from the red cells. In 1847 Zimmerman found in defibrinated blood certain "elementary granules," at first about 1μ in diameter, but gradually increasing in size, and, as he believed, eventually becoming red cells.

The first complete descriptions were give by Schulze, Kolliker,[2] Ranvier, and Bizzozero,[4] and later by many authors.

They are circular or ovoid, colorless, homogeneous, or granular bodies, about 1μ to 3μ in diameter, usually showing no nuclear portion, and staining lightly by both basic and acid dyes. The common impression that they disappear promptly after shedding is true only of the *spindle cells* of the blood of lower animals, while human blood plates are scanty in fresh blood and increase in number upon standing. They are extremely cohesive and apparently of high gravity, and since they collect in masses from which the fibrinous threads of coagulating blood commonly radiate, they are supposed to take an important part in the formation of fibrin.

On account of their uncertain morphology and origin, attempts to enumerate these bodies have not been very successful. In normal blood they may be said to vary between 180,000 (Fusari) and 500,000 (Pruss). Estimates of their numbers based upon the general appearance of the blood indicate that they are specially abundant in afebrile anemias, leukemia, hemorrhages, and that they are deficient in febrile diseases, malaria, and after the administration of various poisons (Afanissiew, Limbeck, Fusari, Pizzini).

The *significance* of the so-called "third corpuscles" of human blood, the blood plates, appears to have been greatly obscured by the discovery in the blood of frogs by Recklinghausen, and of birds by Hayem, and Bizzozero and Torre, of small spindle-shaped elements, which differ from red cells in the absence of hemoglobin and from leucocytes in their simple oval nucleus and non-ameboid protoplasm, and which have been regarded as the homologue of the human blood plates. Löwit[2] classes these spindle cells with leucocytes. Müller believes them to be peculiar elements without relation to either red or white cells. Ranvier regarded them as loosened vascular endothelia. The conclusion of Recklinghausen, Globulew, Schlarewsky, Bizzozero, Hayem, Vulpian, Eberth, and Schimmelbusch, that these cells are analogues of the blood plates, is the chief ground for the belief in the existence of a third corpuscle in human blood.

When the evidence derived from the examination of human blood is considered, it becomes clear that the existence of a third *cellular* body is without proof. Bizzozero's convincing demonstration of the presence of extremely fragile bodies in the blood of the frog's mesentery has naturally never been repeated in human blood, and the dissimilarity between human blood plates and the spindle cells of the frog requires much more cogent evidence of the relation of these two bodies than has yet been furnished. Consequently later investigators have with apparent success endeavored to show *that the blood plates are not cells, but detritus of cells or of plasma.* Howell, Gibson, and Hlava regard the blood plates as fragments of the nuclei of disintegrated leucocytes. Löwit believes many of them to be precipitated globulin particles, and others to be fragments of degenerating leucocytes. On account of their chemical composition Lilienfeld holds that they consist of nuclein, and that they are derived principally from the nuclei of disintegrated leucocytes. Czermak finds them abundantly in the proliferation zones of lymphoid organs, and, with Mondino and Sala, believes them to be fragments of the nuclei of colorless cells.

The studies of Klebs, Engel, Bremer, Wlassow, Arnold,[2] Maximow, and others, have placed beyond doubt the opinion that the chief source of blood plates is by the extrusion from red cells of masses or chains of globular material, which give many of the reactions of the nucleoproteids. While this process may be observed in almost every dry specimen stained by methylene blue (Plate II., Fig. 2), it appears to be favored by proximity to leucocytes, may be accelerated by many reagents, and is probably specially frequent in young red cells.

A recent and one of the most exhaustive studies of batrachian spindle cells is that of Eisen. This observer concludes that the fusiform or spindle cell in *Batrachoseps* arises by extrusion of a part of the mitotic nucleus of the red cell together with some elements of the cytoplasm. This spindle cell is capable of development and of division into two or more plasmocytes (blood plates). The plasmocyte is composed of archosome and three cytoplasmic zones, but has no nucleus. Yet Eisen claims that it is capable of growth by assimilation of food, exhibits phagocytosis and motility, and survives as an independent corpuscle in the blood stream.

There is a somewhat distant analogy between the method of development of the plasmocytes, as described by Eisen, and of the blood plates as observed in human blood, as in both cases these elements represent extruded portions of red cells. It appears very unlikely that the process described in batrachians can be successfully demonstrated in man, while the criteria on which Eisen bases his claim that the plasmocytes are true cells will not satisfy the majority of histologists. At best these elements are only extruded fragments of cells, even in batrachians.

Neither phagocytosis, nor increase in size, nor signs of motility will prove sufficient evidence of the cellular nature of these bodies. Nevertheless, while the majority of blood plates in man must be regarded as extrusions of material from red cells, the comparative study of these bodies suggests that it may be possible to distinguish among human blood plates some which have some other origin and a structure similar to those of batrachians. Recently Deetjen, examining fresh human blood on his salt-agar medium, has described blood plates which exhibited ameboid motion and a structure recalling that of the blood plates of batrachians.

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PART II.

SPECIAL PATHOLOGY OF THE BLOOD.

CHAPTER VI.

CHLOROSIS.

CHLOROSIS is a primary anemia occurring almost exclusively in young women, rarely in young men, resulting from defective hematogenesis and affecting principally the hemoglobin, but, secondarily, also, the number of red cells.

Etiology. The studies of the etiology of chlorosis having established a great variety of predisposing and exciting causes, and rendered it evident that the disease results under very many diverse conditions, have left the essential factor in the evolution of the disease undetermined. On this account some are inclined to regard chlorosis as a symptomatic anemia, holding the peculiar clinical characters of the disease to result from the age and constitution of the individuals in whom it occurs (Kruger).

Regarding the general causes, a number of theories, supported by many important facts, have been brought to light.

Hypoplasia of the arterial system is an anatomical groundwork shown by Rokitansky and Virchow to exist in a certain group of cases. This hypoplasia may affect only the heart and larger arteries or the genital organs as well, or is frequently associated with the other developmental anomalies of the *constitutio lymphatica.* More recent studies (Paltauf, Ortner, Fraentzel) indicate that the congenitally small heart and aorta are more closely associated with the "*constitutio lymphatica*" and with cardiac disease than with chlorosis. This hypoplasia has been found in subjects never suffering from chlorosis, and the majority of chlorotics give no other evidence of such vascular anomalies and make complete and permanent recovery.

Intestinal autointoxication was first suggested as the essential cause of chlorosis by Hoffman, and this theory has been supported on various clinical and pathological grounds by Duclos, Clark, Nothnagel, Bouchard, and many others. Bunge's authority has lent some force to the autotoxic theory, as this investigator holds that the blood obtains iron solely from the nucleo-albumins, the iron from which is combined with sulphur during intestinal putrefaction and thus becomes non-absorbable. In chlorosis the iron administered by mouth combines with the H_2S and permits the normal absorption of the nucleo-albumins.

This theory has been completely set aside by the demonstration by v. Noorden,[2] Rethers, Morner, and Lipman and Wulf, that in chlorosis increased intestinal putrefaction is not commonly present, nor is there increased excretion of the derivatives of Hb. Stockman showed, moreover, that sulphate of iron will cure the disease, while bismuth and some other absorbents of H_2S will not. Intestinal intoxication can, therefore, figure in only a limited group of cases.

v. Hosslin, finding marked increase of iron in the feces of some chlorotic patients, concluded that the blood changes result from small multiple intestinal hemorrhages. Luton accepts this theory.

Functional disturbances of the nervous system have from the first been actively maintained as prominent or exclusive etiological factors in chlorosis. The train of events here involved is variously explained. Murri supposes that there is a vasomotor disturbance arising from the generative organs, and causing changes first in the rate of flow, later in the chemistry of the blood. This theory represents the culmination of opinion as handed down by the older physicians (Trousseau, cf. Lloyd Jones). According to Meinert, the nervous influence arises from irritation of the abdominal sympathetic and results from *gastroptosis*. This observer has brought together very strong evidence to show that in one group of cases the above condition must be an important factor in the coincident anemia. Kruger believes that hypertrophy of the spleen commonly associated with chlorosis (Fuhrer, Scharlau, Chvostek, Clement, Grawitz, Rummo, and Dorri) is an indication of disturbance of function in this organ, which results in an increased destruction of Hb. This disturbance of function is believed to arise through irritation of the sympathetic, in the genital organs, stomach, intestines, etc.

Grawitz believes that the disease is a *vasomotor neurosis*, with secondary changes in the blood consisting in an increase of plasma, which infiltrates the red cells and replaces the Hb. This condition is brought about by a disturbance in the interchange of fluids between the blood and the tissues leaving an excess of fluids in both. That the cells are at the same time deficient in Hb he explains as a result of the imbibition of fluids by their progenitors in the bone-marrow. To support this belief he emphasizes the nervous phenomena of the disease, refers to the fact that the symptoms are often out of proportion to the changes in the blood, which when severe he attributes to complications. He regards the initial loss of weight which many chlorotics suffer in the beginning of treatment as a sign of general reduction of body fluids.

This theory is in accord with many results of recent study, especially those which indicate that a normal amount of iron is maintained in most cases, and that an excess of plasma is one of the essential features of the disease. It appears, however, to give inadequate importance to the loss of Hb, to the changes in the blood of severer cases, and to the therapeutic effects of irritant preparations of iron.

Predisposing Causes. The almost exclusive occurrence of the disease near the establishment of menstruation must prove any theory of etiology inadequate which fails to consider *the age of puberty*, the *female sex*, and the function of *menstruation*. There is abundant evidence to show that *heredity* also is an important factor, as the disease is often observed in the daughters of chlorotic mothers, while a tuberculous family history has been remarkably frequent in some series of cases (Trousseau, Jolly, Bramwell).

Great importance undoubtedly attaches to general *lack of hygiene*, especially to the combination of *poor food* and *mental depression*. Stockman's analyses indicate that the diet of chlorotic girls contains, as a rule, too little iron.

Considering the evidence thus briefly reviewed, the writer concludes that chlorosis is a specific form of essential anemia which results from the association of several factors, of which the two most important and invariably present are (1) a predisposition to the disease, and (2) defective absorption of iron from the food.

The predisposing factors are most pronounced in young girls at the establishment of menstruation, when the unaccustomed losses of blood and the peculiar changes in the nervous system favor the expression of hereditary tendencies and exaggerate the effects of bad hygiene; and when additional elements are thrown in the balance, such as improper food, constipation, gastroptosis, mental depression, etc., the blood begins to suffer in a peculiar way, and clinical symptoms are established which are not seen in secondary anemias occurring in the same subjects. The essential importance of the absorption of iron is indicated by its specific action, but it seems unnecessary to conclude, as Stockman has done, that the food must be deficient in iron, provided *digestion and absorption* are defective. It seems to be an invariable rule that chlorotics do not recover until the appetite improves.

The exact nature of the pathogenic process in the disease remains, however, untouched by these considerations, and it still remains undetermined just how and where the elaboration of Hb is disturbed, whether in the intestinal mucosa (Garrod, Forchheimer), or in the spleen (Kruger), or in the bone-marrow. At present the weight of evidence appears to be almost conclusive that chlorosis results from a functional insufficiency of the bone-marrow, brought about in congenitally predisposed subjects by a series of conditions most often combined in young women at puberty.

Changes in the Blood. Specific Gravity. The specific gravity of the blood in chlorosis is reduced in very uniform ratio with the loss in Hb. From the observations of Devoto, Hammarschlag, Schmaltz, Siegel, Hock and Schlesinger, Menicanti, Stintzing, and Biernacki, it has been shown that this constant relation between specific gravity and Hb-content is somewhat peculiar to chlorosis. It is most uniform when there is no great reduction in cells, but in the severer types of the disease the ratio is less constant.

Jones found the specific gravity of the blood between 1.032 and 1.045 in eighty-seven cases, while that of the serum remained nearly normal, 1.0259 to 1.029. These results were verified by Hammarschlag in thirty cases, in which the blood averaged 1.045, the serum 1.030. The reduction in gravity of chlorosis corresponds, according to Jones, to a physiological diminution occurring in girls at puberty, but not in boys. Grawitz places the specific gravity at the height of the disease between the limits 1.035 and 1.045, and states that below 1.035 some complication is indicated.

The bulk of blood is probably not altered in uncomplicated chlorosis, although Lloyd Jones considers an increase in the volume of plasma to be a very constant feature, and Stintzing and Gumprecht believe that *oligemia* exists in some peculiar cases. The exuded drop is pale in proportion to the anemia, and abnormally fluid.

By the carbonic oxide method Smith calculated that the blood in chlorosis is much increased in volume, finding that while the total oxygen capacity is normal, the percentage capacity for the unit of blood is reduced fully 50 per cent. These results would indicate that the volume of blood is nearly doubled, and they cannot be accepted without confirmation.

The Hemoglobin. The chief alteration in the blood is the LOSS OF HB, which is so far out of proportion to the reduction in red cells that a *low Hb-index* is one of the diagnostic features of the disease.

A contrary opinion is held by Eichhorst and Zumpf, and especially by Biernacki, who, finding a normal or increased percentage of iron in the dried residue of the blood in some cases, believe that *a loss of other albumins and not especially of Hb* is the essential change in the disease. The basis of this dissenting opinion will be further considered later.

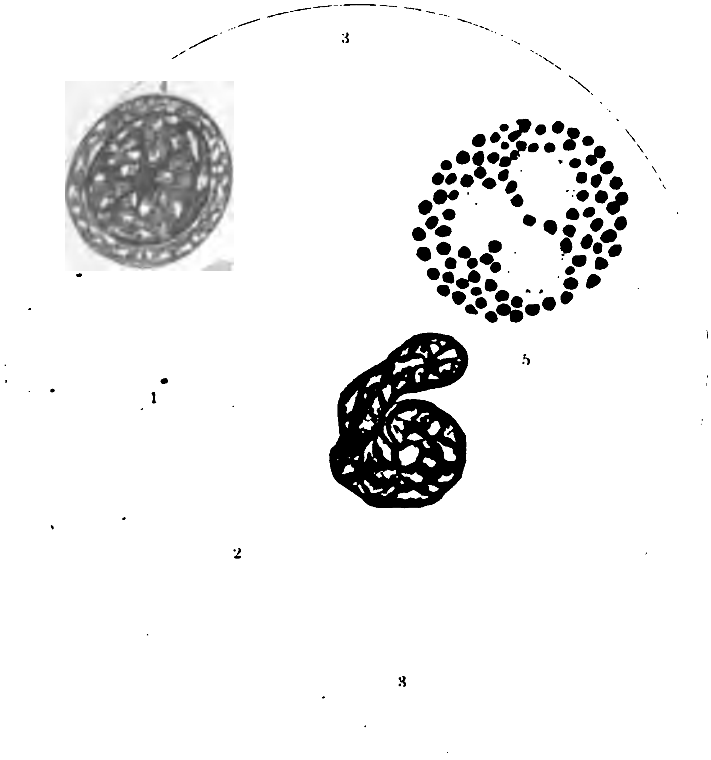
The percentage of Hb obtained by modern instruments has been reported as low as 10 (Bramwell); but when the Hb falls below 20 per cent. there should always be a suspicion of some complicated or secondary anemia.

In cases of average severity the Hb ranges between 35 and 45 per cent., and the Hb-index about 0.5; yet some authors record severe cases with high Hb-index (0.80 to 0.95, Bramwell), although from the details of these reports it is not clear to what this variation from the rule is referable. These cases, in the writer's experience, fall in an intermediate group between chlorosis and pernicious anemia, and are more refractory than the others. Occasionally the Hb-index is very low (0.20, Bramwell).

v. Noorden,[1] from extensive observations, finds that the Hb-index is apt to be somewhat higher in recurrent than in initial attacks, but Romberg could not verify this statement in a group of relapsing cases. The writer has seen illustrations of v. Noorden's rule, and, since increase in size of cells is an indication of chronicity in anemia, believes that the observation is, in general, well founded; yet, as Romberg states, the character of the blood changes is controlled by individual peculiarities in blood formation and by numerous unhygienic conditions.

The red cells are in most cases slightly reduced, although almost invariably much less affected than the Hb; yet not infrequently a

PLATE IV.



Mild Chlorosis. (Eosin and Methylene Blue.)

Fig. 1. Slightly deformed red cells, with enlarged central clear areas, indicating loss of Hb.
Fig. 2. Poikilocyte.
Figs. 3. Normal rouleaux.
Fig. 4. Medium-sized lymphocyte.
Fig. 5. Polynuclear neutrophile leucocyte.

considerable grade of anemia is associated with a normal proportion of red cells. A high count of red cells is a favorable prognostic sign.

In cases of average severity the red cells run from 3,500,000 to 4,000,000, while in severe cases the number falls below 3,000,000. Exceptional examples in which less than 2,000,000 cells were found are recorded by Bramwell, Cabot, and many others, while Limbeck records one case with 1,190,000, and Hayem a minimum of 937,360. In such cases the Hb-index is often comparatively high.

Grawitz emphasizes the importance of the diminution in the volume of the red cells, which he finds without exception is very marked, their volume averaging 20 per cent. instead of the normal 40 to 50 per cent. Since the cells are of normal number and normal size, he refers the diminution of bulk to infiltration with serum which is lost in the process of centrifuging.

In *morphology* the red cells present characteristic changes. In mild cases and those of average severity the sole alteration is a loss of Hb, which causes a progressive widening of the central clear area to be observed in cells rather thickly spread and slowly dried. In all well-marked cases the appearance of such blood in stained smears is characteristic of simple anemia. It is always possible, however, to find a few very small cells and some extremely deficient in Hb, but in the less severe cases these features are not pronounced. (Plate IV.)

When chlorosis is of long standing or of very severe grade, with hydremic plasma, and showing a tendency to relapse, the red cells begin to exhibit marked variations in size and to some extent also in shape. In most cases in which the response to iron is fairly prompt these changes do not affect any considerable proportion of cells. In others in which the response to iron is less certain there is a moderate number of large cells with abundant Hb. (Plate V.)

In no case do these changes reach the grade seen in pernicious anemia; but in a group of cases which do not respond well to iron and are apt to relapse, the variations in size of the cells is considerable, and many show an abundance of Hb. On the other hand, the writer has seen persistently relapsing chlorosis in which all the cells, varying considerably in size, were very deficient in Hb.

Poikilocytosis may be seen in all severe cases of chlorosis, but is seldom very marked.

Other changes occurring with moderate frequency in the red cells of chlorosis are the polychromatic degeneration of Maragliano and that of Gabritschewsky. Granular degeneration is seen to a slight extent in well-marked cases. It was observed by Stengel, White, and Pepper in eleven of eighteen cases.

A *qualitative change* in the Hb in chlorosis is suggested by the results of Henrique's experiments, by which he finds that oxyhemoglobin is much more slowly reduced in chlorosis than in health or in other forms of anemia.

Nucleated red cells are seen in the severer cases of chlorosis under various conditions. It is said that when present they are subject to periodical variations constituting the "blood crises" (Neudorfer)

Most writers have found them too scanty even in severe cases to permit a full verification that "blood crises" are of frequent occurrence, and this common experience is in accord with the theory that functional insufficiency of the marrow is essentially connected with the pathogenesis of the disease. Their appearance is somewhat favored by rest in bed and large doses of iron, which, as Hoffman has shown, induce hyperemia and cellular hyperplasia of the red marrow. The writer has seen an increased number during the leucocytòsis of an intercurrent pneumonia.

The nucleated red cells of chlorosis are of the normoblastic type, and while Neudorfer and Hammarschlag report finding megaloblasts in severe relapsing cases, the exact nature of these cases is not entirely clear. Their observations require confirmation.

Chemistry. The ALKALINITY of chlorotic blood has been found by the majority of investigators to vary within normal limits (Kraus, Steindler, Limbeck) or in some cases to be slightly increased (Graeber, Peiper, Rumpf). v. Jaksch, however, found it diminished. Burmin, using Landois' method, which gave a normal alkalescence of 0.182 to 0.218 gramme of NaOH, found the alkalescence of chlorotic blood to range between 0.128 and 0.200 gramme NaOH. The alkalescence varied with the red cells, and in six of nine cases the increase of these cells following the use of iron was accompanied by a corresponding increase in alkalescence.

The ISOTONIC TENSION of the red cells was found by Limbeck in two cases to be very low (0.38 to 0.4 per cent. NaCl), their resistance, therefore, being correspondingly increased. Aporti tested the red cells in chlorosis by Viola's method, which determines the least, the medium, and the greatest resistance. The least resistance was much below normal—*i. e.*, some cells dissolved very readily, but not more so than in secondary anemia. Iron at first rendered some cells even less resistant, but after some use the point of least resistance rose rapidly.

The COAGULABILITY of chlorotic blood is notably greater than that of other forms of anemia of equal grade—a rule which accords with the fact of the preservation of the albumins of the plasma (cf. Biernacki).

The PROPORTION OF WATER in chlorotic blood increases from the normal (77 per cent.) to 80 to 90 per cent., and the dry residue falls from 22 or 36 per cent. to 19 per cent. with 60 to 70 per cent. Hb, or as low as 10.64 per cent. of residue with 25 per cent. Hb. In this residue the chlorides, as in all hydremic states, are in most cases considerably increased. Phosphorus is moderately reduced, and iron is deficient in the well-marked cases only (Biernacki). When the Hb is below 70 per cent. the reduction in iron is rather uniformly proportionate with the loss in Hb.

ALBUMINS. Since the specific gravity of the serum is but slightly altered in the average case of chlorosis, the loss of substance must affect principally the red cells. It is very generally agreed that, as shown by Becquerel and Rodier, the principle chiefly affected is the Hb; but Biernacki found a normal proportion of iron in the dry residue of some milder cases of chlorosis, and has concluded that other albuminous constituents may suffer more than the Hb. In Biernacki's analyses of more severe cases, however, a marked and uniform reduction of iron is recorded.

In all the severer cases the serum is poor in albumins, but markedly so only in very severe cases. The hydremia of the serum in chlorosis is relatively far less than in simple anemia, *e.g.*, post-hemorrhagic (Grawitz). This rule is found by Stintzing and Gumprecht to hold only when the Hb is between 50 and 80 per cent., as illustrated in the following table:

	Hb.	21-30	31-40	41-50	51-60	61-70	71-80	81-85
		Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Dry residue.	Simple anemia.	13.4	12.0	13.3	14.5	16.1	18.5	....
	Chlorosis.		12.5	13.4	15.8	18.3	18.8	19.0

Regeneration of the Blood in Chlorosis. Red Cells and Hb. Hayem divided the process of regeneration of the blood in chlorosis into two periods: *one* in which the red cells are increased in number by the appearance of many small pale and deformed cells, followed by a *second* in which these new cells gradually acquire normal characters. Similar observations have been made by the majority of recent observers, who have regarded the small cells seen in relative excess in well-marked cases as being recently formed. On the other hand, Laache, Stintzing and Gumprecht, Reinert, and Graeber find that the increase in Hb usually outstrips that of the red cells.

More recent studies in this field have thrown some light on the difficulty, Romberg showing that with a slight decrease of cells the Hb increases the more rapidly, while in cases with marked decrease in cells these increase first and more rapidly but less uniformly than the Hb. In some severe cases Romberg noted a slight initial diminution in cells following the use of iron.

Grawitz finds that the first change observed in the blood of severe cases is an increase in specific gravity (1.035 to 1.042) with simultaneous increase in the number of red cells—changes which he refers exclusively to diminution in the volume of plasma. Later the new formation of cells and the restoration of the Hb begin, and both progress usually with equal rapidity.

Schaumann and Willebrand have recently fully demonstrated that in moderately severe cases, under iron, there is a progressive increase in the average diameter of the red cells, resulting from the disappearance of undersized corpuscles, while the number of large cells becomes considerable. The authors feel justified in concluding that the progress of regeneration is not marked by the appearance of many small pale cells, which they believe are degenerated forms. In Romberg's one hundred cases, in all of which the cells were below 4,000,000, the average *duration of treatment* was 26.5 days, the Hb increasing 9.9 per cent. and the red cells 430,645 every ten days. The best results followed the use of ferri. carb. sac., but they did not use Blaud's pills.

Distinct polycythemia has in many instances been found to follow recovery from chlorosis. Schaumann and Willebrand report 7,400,000

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2. The red cells are below 4,000,000, but there are no marked changes in the shape and size of the cells; the Hb-index is very low; the patients are prostrated, but with iron usually recover promptly, and the anemia does not often relapse.

3. The red cells are markedly reduced (below 3,000,000 to 3,500,000); there are considerable changes in shape and size, the Hb-index varies, but is usually very low; the patient responds more slowly to iron, and the anemia often relapses.

(*b*) A group of cases of idiopathic anemia common in young women has long been recognized, in which the symptoms resemble those of refractory chlorosis, but which in some other respects are quite different. Delafield states that they are chiefly distinguished by their failure to react to iron. A chronic course with relapses is commonly observed, but a change to distinct pernicious anemia rarely occurs. The blood of such subjects shows a marked reduction in red cells, commonly between 2,000,000 and 3,000,000, and marked variation in size of the cells, a considerable number of which have increased Hb. The Hb-index may be very low, but is apt to be higher than in the average case of chlorosis. The lymphocytes are commonly in excess.

It is possible that some of these cases are much more anemic than the ordinary blood examination indicates, because complicated by oligoplasmia, as suggested by Lloyd Jones and Biernacki.

(*c*) **Pseudochlorosis** is a term employed by various recent writers to denote a group of cases in which the external symptoms of chlorosis are present, but in which, from the examination of the blood, it is found that the Hb, number of red cells, percentage of dry residue, and usually the proportion of iron are nearly normal. Several explanations have been offered regarding these obscure cases.

Lloyd Jones believes that they are cases of anemia with *oligoplasmia*, in which the real deficiency of Hb and cells is masked by diminution in the quantity of plasma. He offers no chemical analyses to support his view. Biernacki has studied this condition in detail, and as a result of clinical observations and chemical analyses he concludes that in cases of anemia there is a condition of oligoplasmia wth hydremia of both cells and plasma, so that while the blood shows normal Hb, yet the swollen cells are deficient in this principle, and both cells and plasma are deficient in albumins. *In short, Biernacki claims to have demonstrated that it is possible to have hydremic blood which yet gives a normal percentage of Hb and a normal number of red cells.* Biernacki points out that two of the eight original cases examined by Becquerel and Rodier, and two cases reported by Stintzing and Gumprecht, showed slight hydremia, a nearly normal percentage of iron, but well-marked symptoms of chlorosis. He protests against calling such cases pseudochlorosis, believing that they are genuine examples of the disease, and claims that the essential element in chlorosis is not the loss of Hb and iron, but that the disease is a *neurosis* with *hydremia, and with or without loss of iron.*

If Biernacki's results obtain confirmation, which they have not yet secured, much needed light may be thrown upon a large group of cases in which the symptoms of anemia are associated with apparently normal blood. Such cases occur usually in young women, are often encountered in gynecological clinics because of amenorrhea, and have been fully described, especially by Biernacki and Romberg. It is possible, also, that a similar explanation may apply to the cases of pseudochlorosis described by Stintzing and Gumprecht and others, and very commonly encountered in tuberculous subjects.

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CHAPTER VII.

PROGRESSIVE PERNICIOUS ANEMIA.

THIS term is applied to a peculiar disease of the blood resulting from defective hematogenesis and excessive hematolysis, characterized by severe and usually fatal anemia, peculiar morphological changes in the red cells, and by characteristic changes in the bone-marrow. Although excited by a number of well-recognized conditions, progressive pernicious anemia differs from secondary anemia in the peculiar changes in the red cells, in the lesions of the marrow, in its tendency when once established to progress to a fatal issue, and in the fact that the anemia is apparently out of proportion to the exciting cause, while very often no cause whatever can be assigned.

The disease has often been described as *primary* pernicious anemia, but since typical examples have been positively traced to the effects of intestinal parasites it becomes necessary to admit that this condition of the blood is not always of cryptogenic origin. Nevertheless, its pathological features remain so distinctive as to justify the common belief in the peculiar nature of the disease.

Historical. A review of the early literature on pernicious anemia, as collected by Eichhorst, Musser, and Stockman, shows that while the disease had previously been described by observers in many countries, its symptomatology, gross pathology, and separation from chlorosis and secondary anemia were not clearly stated until Addison in 1855 published the description of cases observed by him clinically and examined at autopsy during the previous decade.

Of the earlier reported cases those of Andral (1821) in France, of Coombe (1823) and Hall (1843) in England, and of Channing (1849) in America were so clearly described as to leave no doubt that fatal idiopathic anemia was fully recognized by some observers at that early time. Channing and other Boston physicians had notes on such cases as early as 1832, and his descriptions of cases in 1849 were sufficiently complete to warrant their acceptance as referring to genuine examples of the disease.

Lebert in Zurich had apparently recognized and partially described the disease as early as 1854; but his pathological reports were too scanty to demonstrate the probable specific nature of the malady, which he did not claim until 1858, although recognizing it as a fatal form of anemia. Long before this, as shown by Lepine, some prominent text-books had called attention to the occurrence of fatal cases of anemia, considering them usually as instances of fatal chlorosis (Piorry, Wunderlich, Schonlein, Rokitansky, Canstatt).

Addison, however, offered more abundant clinical and pathological evidence to show that the disease is of idiopathic origin, fully distinguishable from secondary anemia; but he supposed that the fatty changes were the cause, not the result, of the anemia. In 1857 Wilks' description of seven cases was very clear, and indicates that the condition had then become well identified at Guy's Hospital and was distinguished from secondary anemia and, by the examination of the blood, from leukemia.

In Germany there were fragmentary reports of probable cases by Zenker, 1856; Wagner, 1859; and Grohe, 1861; until Biermer in 1868 collected a series of cases observed in Zurich during previous years, and gave such a systematic description of the clinical symptoms as to attract general attention to the malady as a special form of anemia. Biermer failed to add to the knowledge of the pathology of the disease and paid little attention to the blood; and when Immerman contributed important critical studies, in 1874 to 1877, interest in the microscopic examination of the blood concerned chiefly the proportion of leucocytes.

The early observers, especially Wilks, had noted the marked oligemia, hydremia, and feeble coagulation of the blood at autopsy, and by microscopic examination during life had demonstrated an excessive loss of red cells and a small proportion of leucocytes; but for many years no further advance was attempted in this field. In 1873 Ponfick found a relative increase of leucocytes in one case, probably from antemortem leucocytosis, and noted a greenish tinge of the serum.

In 1876 Quincke described the appearance of microcytes and poikilocytes in several cases, while Eichhorst, at the same time, noted the absence of rouleaux and the increase of Hb in some microcytes, cells which he regarded as pathognomonic of the disease.

By this time Bizzozero's and Neumann's demonstration of the function of the bone-marrow in blood formation began to turn attention to this tissue, Pepper (with Tyson) making the first contributions in a new field in 1875. Pepper found uncertain evidences of a cellular hyperplasia in some portions of the marrow, that of the radius appearing paler than normal, while that of the sternum was quite red.

A great advance was made when Cohnheim in 1876 presented a brief communication on the changes in the bone-marrow in a case of pernicious anemia. He reported finding an unusual hyperplasia of the red marrow, with complete atrophy of fat cells, increase of myelocytes, large and small, absence of normal red cells, and the presence of megalocytes, microcytes, and large numbers of megaloblasts. In the blood of the *vena cava* he found a few large nucleated red cells, but had failed to find them in the peripheral blood during life. Cohnheim expressed the opinion that the essential element in the malady was a primary disease of the marrow affecting the development of the red corpuscles, leading to production of megalocytes at the expense of smaller properly functionating cells, the whole constituting *a reversion to the embryonal type of blood formation.* Cohnheim thus completed the chain of pathological evidence that was required to establish the distinctive nature of the disease, and demonstrated the importance of closer study of the blood and marrow as the seat of the primary lesion. The further study of the marrow was taken up by Osler and Gardner, Neumann, Litten and Orth, P. Grawitz,[2] and many others, whose results have shown that the study of the changes in the marrow gives the deepest insight into the essential process of the disease.

The first enumeration of red cells was reported by Sorensen, who, using Malassez's method, found in one case only 470,000 cells; by Lepine,[2] who, with Hayem's method, reported a reduction to 909,000 and 378,750; and by Ferrand, who found 500,000 red cells and 10 per cent. of Hb. Of morphological changes an increase in size of the red cells during the course of the disease was noted by Lepine; a slight brownish tinge by Striker; the presence of ameboid processes by Scheby-Buch; the oval form and absence of rouleaux by Bradbury. In 1876 Quincke described the appearance of microcytes and poikilocytes in several cases. Eichhorst (1878) found the most striking characteristics to be the large size, pallor, and scarcity of the red cells; yet all these characteristics Eichhorst had seen in a case of gastric cancer, whence he maintained that the blood of pernicious anemia showed no single pathognomonic sign. While Quincke had observed that many microcytes seemed to contain an excess of Hb and that the blood of pernicious anemia was more highly colored than the number of red cells warranted, it was Hayem[2] who first demonstrated that an increased Hb-index is characteristic of the disease, while Laache showed this feature to be referable to the presence of many megalocytes with increased Hb.

By 1880 there were only two reported cases in which nucleated red cells had been found during life, when Ehrlich, examining stained dry preparations, claimed that while nucleated red cells were found in all cases of severe anemia, megaloblasts were seen exclusively in progressive pernicious anemia. Only in recent years has this claim been shown to be but partially valid. In 1891 Luzet first reported the discovery of mitotic nuclei in megaloblasts from the blood of a child, the occurrence of which has since been shown to be a nearly pathognomonic sign of the disease in adults. With the observations on polychromatic degenerative changes in red cells these lesions were found to be specially distinct in pernicious anemia. In one of v. Noorden's dissertations was first mentioned the occurrence of basic staining granules in the bodies of megalocytes, which was later verified by Askanazy and interpreted as evidence of karyorrhexis.

The contributions on etiology which have steadily accumulated from many sources, and the progress of the knowledge of the chemistry of the blood, chiefly through the work of v. Jaksch, Grawitz, Dieballa, and others, will be considered under those topics.

General Etiology. After the demonstration of striking and peculiar changes in the blood and marrow it was generally accepted that progressive pernicious anemia is of idiopathic origin, although Biermer enumerated a list of exciting causes which appeared to have been present in his cases, viz., insufficient food, bad hygiene, prolonged diarrhea, repeated hemorrhages; yet it appeared that the assignable causes were inadequate to account for the result, and it was felt that the disease could in no respect be classed with the ordinary cases of secondary anemia such as follow carcinoma, nephritis, hemorrhages, etc.

The first retrenchment of this view was rendered necessary by the discovery of typical cases of the disease referable to the presence of intestinal parasites (*bothriocephalus*), and which recovered promptly after expulsion of the worms. These cases showed that there is not always a cryptogenic factor in the etiology of the disease, and that the presence of the typical blood changes does not necessarily imply a fatal course of the anemia. Likewise the changes in the marrow were for a time robbed of much of their pathognomonic value by the report that very similar changes are established in some cases of multiple hemorrhages (Neumann), tuberculosis (Litten), and gastric cancer (Eisenlohr). Further, the changes in the blood have now been shown to follow syphilis (F. Müller),[1] malaria[2] (Bignami,[3] the writer); atrophy of gastro-intestinal mucosa (Fenwick, Brabazon, Henry, Osler, Nothnagel, Stengel); typhoid fever (Quincke, Rosenstein, P. Grawitz); prolonged diarrhea (Wilkes, Frankel, Stricker); myeloid sarcoma (Litten); in each of which conditions it is occasionally impossible to say that the cause is inadequate to the result, and there remain only the acute cases without demonstrable origin but referred by some to intestinal intoxication and the cases that follow pregnancy, which can still be held as of cryptogenic nature.

Scope of the Term Progressive Pernicious Anemia. In view of this present state of the subject two plans of classification of the severe anemias of the Addison-Biermer type have been suggested by Birch-Hirschfeld and Grawitz.

Birch-Hirschfeld claims that there is no good reason to separate on etiological grounds alone cases of severe anemia of cryptogenic origin from those like the anemia of bothriocephalus, in which an adequate cause is apparent, for both show the same anatomical evidence to prove that the pathological process in each is identical. He would, therefore, class together all severe anemic conditions, without regard to origin, in which there is degeneration and loss of red cells, increased destruction of tissue-albumins, and fatty changes in the viscera, and in which the chief factors are excessive destruction and defective formation of red cells.

Against this proposition Grawitz urges that it is clinically inapplicable, because fatty changes in viscera can be recognized only after death; that it classes together anemias arising from such diverse causes as cancer, malaria, sepsis, ankylostomiasis, etc., and that increased destruction of albumins is not a constant feature of the disease, although present in some forms of milder secondary anemia. Grawitz, therefore, proposes to throw out of the category of progressive pernicious anemia those cases in which the etiology is known, *e. g.*, *bothriocephalus*, and to retain in this class only those cases in which the most thorough examination of the patient fails to show any primary disease.

This suggestion accords with the view of those who fail to see in the histological changes in blood and marrow evidence of a peculiar condition interpreted by Cohnheim, Ehrlich, and many others, as a reversion to the embryonal type of blood formation.

The writer, having failed to find from any source a controversion of the view that the changes in the blood and marrow described in

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pernicious anemia result from a peculiar pathological process which is essentially different from that seen in the majority of secondary anemias, must accept the opinion that when the blood contains megaloblasts and a considerable proportion of megalocytes with increased Hb, while the lymphoid marrow shows marked hyperplasia of peculiar type, the condition should be called progressive pernicious anemia, without regard to its immediate exciting cause. The essential process in the disease is a reversion of the marrow to a type of blood formation which in some respects resembles the embryonal; and it would seem to make very little difference how various the exciting agents may be, whether syphilis, malaria, ankylostomiasis, or gastro-intestinal infection (Hunter), provided they all initiate the same process and stamp the anemia with certain self-perpetuating tendencies not seen in other conditions.

Special Etiology. Progressive pernicious anemia is, as shown by its history, a disease of very general distribution and comparatively frequent occurrence. In the Berlin City Hospital, Lazarus found 274 cases recorded in ten years, or 2 per cent. of the admissions. It is rather less common in New York hospitals, but consultants in the city see from six to twelve cases yearly.

The female *sex* is considerably more subject to the disease than are males, owing probably to the predisposing influence of pregnancy (Müller, Ehrlich).

The 240 reported cases collected by Ehrlich were distributed between the *ages* as follows:

	Cases.		Cases.
1 to 10	1	40 to 50	47
10 to 20	22	50 to 60	33
20 to 30	61	60 to 70	7
30 to 40	67	70 to 80	2

Monti and Berggrun collected sixteen cases in children, of which five occurred between the first and fifth years, and nine between the fifth and fourteenth years. Ehrlich hesitated to accept these as genuine cases on account of the uncertain significance of changes in the blood in early life.

Intestinal Parasites. That the presence of intestinal parasites is frequently responsible for grave forms of progressive pernicious anemia was first demonstrated for *ankylostoma duodenale* by Griesinger in 1854, and has since been amply attested. The work of Perroncito on *ankylostoma* was followed by the demonstration by Hoffmann, in 1885, that infection by *bothriocephalus latus* is much more frequently associated with very grave types of anemia indistinguishable from the idiopathic progressive disease.

Oxyuris was found associated with pernicious anemia first by Runeberg[2] in 1887, and ascaris by Demme in two fatal cases in children. In all of these infections the grade of anemia may vary from the simple chlorotic type to the very rapid and fatal pernicious form. The presence of eosinophilia in any case of pernicious anemia should suggest a search for one of these intestinal parasites.

Repeated Hemorrhages. That genuine progressive pernicious anemia may follow repeated hemorrhages has been claimed by many observers who have not insisted upon Ehrlich's signs in their diagnoses (Habershon, Quincke, Scheperlen, Finney, Greenhow, Stockman). In recent years reports of genuine cases of this origin have not been so numerous, yet Ehrlich and Lazarus accept as a fact their frequent occurrence from this cause. The writer has not seen any cases directly referable to hemorrhage.

It is probable that the genuine cases observed after pregnancy are partly the result of the losses of blood during and after parturition. Stockman goes so far as to urge that all cases now called idiopathic are the result of repeated minute intestinal hemorrages, in which opinion he stands unsupported.

While granting, as does Ehrlich, that the disease occasionally follows repeated hemorrhages, it still remains true that the great majority of chronic posthemorrhagic anemias follow the type of secondary anemia with very low Hb-index, and with leucocytosis.

Pregnancy was one of the conditions first recognized as a prominent exciting cause of pernicious anemia, the most numerous cases (twenty-nine, 35 per cent.) of those collected by Eichhorst (1878) coming under this heading.

The genuineness of some of these cases may now be doubted, and at any rate the patients suffered from a variety of severe symptoms not at present regarded as belonging to the disease, so that Lazarus classes many of them as examples of secondary anemia and exhaustion. In others, however, the pregnancy was uneventful except for the spontaneous development of severe anemia ending fatally before or after parturition, so that Leiden ascribed to pregnancy a special *nervous influence* leading to fatal anemia, and *Birch-Hirschfeld* assumed that placental toxins affect first the plasma and later the red cells. These theoretical considerations have been rendered unnecessary by the gradual disappearance from the literature of cases of progressive anemia following pregnancy. [illegible] one case of this description in [illegible] (Lazarus [illegible]). At Sloane Maternity Hospital from [illegible] the writer saw many cases of severe anemia, but none of the progressive pernicious type. Ahlfeld, in a wide experience, [illegible] following pregnancy [illegible]. The [illegible] of severe anemia following pregnancy [illegible] type, and, as Ehrlich concludes, [illegible] pregnancy any peculiar influence in the origin of the [illegible].

Syphilis. That syphilis [illegible] which the morphological changes [illegible] those of progressive pernicious anemia [illegible] attested by the reported cases of Lazarus [illegible]

In children suffering from hereditary syphilis [illegible] the size of red cells, their increase of Hb [illegible] megaloblasts seem to [illegible] syphilis figures as an exciting cause of progressive pernicious anemia (Loos, Lazar); yet the reports in recent literature [illegible] ened the view that syphilis may lead directly to this peculiar type of anemia. The changes in the blood seen in the great majority of cases, although often severe, seem to follow the type of simple secondary anemia. Consequently Ehrlich and Lazarus doubt the existence of the disease as a result of syphilis.

The writer has seen excess of deeply staining megalocytes and megaloblasts in several cases of moderate severity shortly after florid syphilis, both in adults and infants, and has come to regard the differential diagnosis of severe secondary anemia in syphilitic subjects from progressive pernicious anemia as one of the most difficult fields in blood analysis. From experience at the autopsy-table it has been very apparent, however, that most cases showing advanced or active tertiary lesions have shown types of secondary anemia. The subject requires much more extensive clinical and pathological observation.

Malaria. Although there are in the literature numerous cases of so-called pernicious anemia referred to malarial infection, the fact that the typical changes of the disease may be established as a result of chronic malaria was not demonstrated until Bignami and Dionisi reported their study of the marrow of fatal cases. Here were found the signs of megaloblastic hyperplasia in distinct form. Among the writer's Montauk cases there were no less than nineteen in which the blood showed a majority of megalocytes with increased Hb, and a considerable number of megaloblasts, and in one of these the lymphoid marrow was found markedly hyperplastic, with excess of megaloblasts, and an enormous number of young estivo-autumnal parasites. In all these cases the condition had become established within eight to ten weeks from the beginning of the infections, but the malarial element had doubtless been aided by exposure and poor food.

The frequency of these cases among malarial subjects and the fact that the bone-marrow suffers in a peculiar way in many cases of estivo-autumnal infection are considerations which bear directly on the myelogenous origin of pernicious anemia.

Typhoid Fever. Quincke and Rosenstein each report the transformation of the cachexia of typhoid fever into pernicious anemia, but in neither case do their reports show the presence of typical changes in the blood, both falling in the class of severe secondary anemia.

Gastro-intestinal Disorders. The largest group of cases, and those which from the first have given most support to the theory of the cryptogenic origin of the disease, have been referred to disturbance of the gastro-intestinal tract. A certain number of these cases have shown at autopsy well-marked lesions of stomach or intestines which have been deemed a sufficient cause of the anemia, while in others the stomach and intestines have been found normal, and the theory of intestinal intoxication has been elaborated, principally by Hunter, to account for the anemia. Hunter emphasizes the fact that Addison's anemia, according to its original description, is essentially a cryptogenic disease in which the autopsy reveals none of the causes assigned by Biermer and others. This group of cases he separates from all others as true Addison's anemia, and for them he finds a peculiar etiology in an infectious glossitis and gastro-enteritis.

1. LESIONS OF GASTRO-INTESTINAL TRACT. Chronic gastritis or enteritis with fatty degeneration of the secreting cells of the peptic and intestinal glands, followed by atrophy and sclerosis of the mucosa, have been demonstrated by Ponfick, Nothnagel, Fenwick, Levy, Brabazon, Nolen, Eisenlohr,[2] Osler, Ewald, Martius, Koch, Stengel, and Strauss.

Ewald found this condition of the gastric or intestinal mucosa in all his subjects examined at autopsy, and believes that such changes, which he calls *anadenia*, are responsible for all "idiopathic" cases; but Immerman and Quincke had previously shown that *anadenia* is not found in all cases of clearly protopathic origin, and this claim has been verified by recent observers. The writer has secured excellently preserved peptic and intestinal glands and intact lymphoid structures from an acute case showing no visceral lesions to which the anemia could be referred, and Eisenlohr and Martius have reported extensive atrophy of the intestinal mucosa in cases showing no gastro-intestinal disturbance and slight anemia.

Stenosis of the pylorus as a result of annular carcinoma or of chronic gastritis, with extreme atrophy and contraction of the stomach, has been demonstrated in a considerable number of cases (Israel, Ehrlich, Renvers).

In two of the writer's autopsies the stomach was markedly contracted, the pylorus was tightly stenosed, admitting a lead-pencil with difficulty; the mucosa showed the changes of well-marked chronic gastritis, but the constriction of the pylorus was the result of chronic productive inflammation and not of carcinoma. These cases lasted two and four years, with intervals of improvement, but finally died with typical blood changes and without marked emaciation.

Other cases with annular or circumscribed *carcinoma of pylorus* have been reported as showing typical changes in the blood. The writer has not yet seen any case of cancer of the stomach in which the lesions in the blood were indistinguishable from the classical type of progressive pernicious anemia. Lazarus reports genuine cases following small pyloric cancers only.

A "*neurotic atrophy*" of the intestinal mucosa has been suggested as an etiological factor by Banti, Jurgens (1882), Blaschko (1883), and Sasaki (1884), who found parenchymatous degeneration of ganglion cells in Meissner's and Auerbach's plexuses; but Scheimpflug has shown that no such important deductions were warranted from the changes demonstrable at that time in sympathetic ganglia, since identical changes occur in many other conditions. Peculiar eosinophile globules have been described by Lubarsch, Koch, and Sasaki in the atrophic adenoid tissue of chronic gastritis, and these bodies have been regarded as pathognomonic of this condition and of significance in the anemia; but Hammarschlag has seen them in gastric cancer and the writer in the normal stomach at autopsy one hour after death from ether.

The mesenteric lymph nodes have been found hyperplastic, in one instance caseous, by Eichhorst, Quincke, and others. This condition was present in some of the writer's autopsies, but not in all, while in a case of profound secondary anemia, probably of syphilitic origin, nearly all the abdominal l nodes were enlarged and caseous, but there were no megaloblastic cha the blood.

It is, therefore, obvious that all the gastro-intestinal lesions thus far described in cases of progressive pernicious anemia have been seen in other conditions, and that none of them can be regarded as specific lesions in the disease. That many of the above lesions, however, especially the marked atrophic conditions, may act as occasional exciting causes of progressive pernicious anemia, as of secondary anemia, seems equally probable; yet not a few observers regard them all as secondary to the anemia or as unimportant associated lesions, while Faber and Bloch believe that the atrophy of the intestinal mucosa described in many cases is always a cadaveric alteration.

2. FUNCTIONAL DISTURBANCES OF GASTRO-INTESTINAL TRACT. The prominence of the gastro-intestinal symptoms of the disease has from the first directed special attention to the study of functional disturbances in the alimentary tract.

The reports of Sandoz were apparently the first to seriously strengthen the impression that some of the most typical cases of pernicious anemia are of intestinal autotoxic origin, this observer finding that apparently genuine cases were sometimes cured by vigorous gastric lavage, enteroclysis, and the administration of intestinal antiseptics and laxatives. This observation has since been fully verified, and the opinion has steadily grown that the most frequent, if not the essential, cause of progressive pernicious anemia is found in a peculiar toxemia of intestinal origin, with or without organic lesions of the mucosa. The evidence supporting this opinion has accumulated from many sides.

The result of intestinal antiseptic treatment has steadily pointed in this direction. The observations on bothriocephalus anemia have recently given significant evidence to a similar effect (q. v.). Signs of increased intestinal putrefaction have been noted in the excessive indicanuria of the disease (Senator, Müller, Brieger, Hennige, Grawitz, Schaumann), and in the presence of cadaverin and putrescin in the urine of certain cases (Hunter). In a very acute case examined at autopsy by the writer the odor of H_2S from the intestines was very intense, but in other cases there was very slight evidence of this putrefactive product. Grawitz[4] has recently emphasized the belief that intestinal toxemia from decomposition of albumins and failure of absorption of albumins acting in subjects predisposed to anemia are the probable causes in the cryptogenic variety of pernicious anemia.

3. SPECIFIC INFECTION OF THE ALIMENTARY TRACT. Hunter's studies stand practically alone in this field. His conclusions are, briefly, that true primary (Addison's) pernicious anemia is a distinct pathological condition characterized by excessive hematolysis, occurring chiefly in the portal system and induced by infection of various structures along the alimentary tract with the streptococcus.

Reviewing the knowledge of the pathological changes in the blood and marrow, spleen, and lymph nodes, Hunter concludes that there is no known characteristic lesion of the disease, although the high Hb-index of the blood he regards as very important and an almost pathognomonic sign. Turning to the liver he finds in the studies of Peters proof that in the anemia of wasting diseases there is no markedly excessive deposit of iron in the liver, and finds that in no disease bearing any clinical resemblance to pernicious anemia does the deposit of iron in the liver approach in any degree that characteristic of pernicious anemia. Moreover, this deposit is largely limited to the outer two-thirds of the lobules, a position in which the scanty deposits of other conditions (extravasation of blood, chronic congestion) are never limited. From the comparison of chemical analyses he finds that the liver in pernicious anemia contains on the average seven times as much iron as in any other diseases attended with anemia, while the iron-content of the spleen is not notably increased. He therefore concludes that pernicious anemia consists essentially in excessive hematolysis, occurring principally in the liver. Why Hunter limits the blood destruction to the portal system is not entirely clear, but he reasons as follows: Malaria, paroxysmal hemoglobinemia, and poisoning by pyrogallic acid cause general hemoglobinemia and hemoglobinuria, with deposits in the liver unlike those of pernicious anemia, while in this disease and in poisoning by toluylendiamin there is never any Hb in the urine, but in each case the iron in the liver is found in the same position. Since toluylendiamin exerts a specific stimulating action on the liver cells, he believes the poison of pernicious anemia must be directed especially against the liver, and hence be found principally in the portal system, a source which is further indicated by the frequency of gastro-intestinal symptoms.

Hunter's studies are chiefly important in the demonstration of the peculiar behavior of the liver in ridding the blood of iron, but the theory of portal hematolysis by intestinal intoxication, while doubtless strongly suggested by his results, is still far from demonstration. He has recently attributed to bacteria from decaying teeth, streptococci, and to chronic infectious glossitis and gastro-enteritis from this source, the essential part in the etiology of the disease; but this claim has not been received with much favor on account of observations like that of McCrae, who found in forty cases of pernicious anemia that oral sepsis was less frequent than in the routine average of hospital cases.

Although extensive deposits of iron in the liver and the limitation of the pigment to the outer portions of the lobules have since been shown to occur in other diseases (Russell, Hindenlang), and cases of pernicious anemia have been reported in which no excess of iron was found in the liver (Ransom), Hunter has demonstrated characteristic if not pathognomonic features in the pathological anatomy of the disease.

Myelogenous Origin. There are recorded a moderate number of observations which suggest that progressive pernicious anemia may result directly from lesions affecting the normal physiology of the bone-marrow. Fede, Quincke, Krieg, and P. Grawitz have described cases of sarcoma of the bone-marrow with the changes of pernicious anemia in the blood. Litten (1877) reported a case of pernicious anemia followed by leukemia in which there were multiple abscesses in the bone-marrow, but the diagnosis of pernicious anemia appears doubtful. Waldstein in 1882 saw a similar case in which the marrow was the seat of a peculiar change said to have resembled in structural details a chloroma found in the mediastinum.

Grawitz believes that the severe anemia sometimes following infectious processes is to be referred to changes in the marrow instituted during the course of the infection. The frequent occurrence of pernicious anemia after malaria appears to have similar import.

Nervous Origin. The prominent participation of the *nervous system* in the anatomical lesions of the disease has often raised a suspicion in many minds that some of these lesions might be primary. Except in the case of the abdominal sympathetic, none of the lesions have been long claimed to hold such a position in the pathology of the disease, and at present it is generally accepted that the central nervous system suffers only secondarily from the anemia or as the result of associated conditions.

Likewise the functional disturbances of the nervous system cannot be claimed to act as more than somewhat distant predisposing causes, although McKenzie and Curtin have referred to certain cases as having originated directly from nervous shock.

Infectious Origin. Pernicious anemia has not escaped classification among infectious diseases: by Klebs, who claimed to have observed flagellate bodies in the fresh blood; by Frankenhauser and Petrone, who reported the isolation of *leptothrices* in several cases; by Bernheim, who isolated a bacillus from the blood of one case after death; and by Perles, who observed highly refractive, very actively ameboid bodies in several cases; and finally by Hunter.

Histological Changes in the Marrow. Cohnheim's original report on the changes in the marrow of a case of pernicious anemia described the presence throughout the shafts of the long bones of lymphoid marrow which contained no fat cells, but was composed of a small number of colorless myelocytes and of many red cells, most of which were nucleated and of unusually large size. This observation was verified by many later investigators, especially by Rindfleisch, Ehrlich, Muir, Müller;[2] but its importance was greatly obscured by the reports by Neumann, Litten, Grohe, and others that chronic diseases causing cachexia also lead to a hyperplasia of red marrow in the shafts of long bones. Neumann and Litten and Orth claimed also to have induced identical changes in the marrow by subjecting animals to repeated hemorrhages. In 1886 Geelmuyden claimed that the hyperplastic marrow in such chronic diseases does not differ in structure from that of normal lymphoid marrow except in regard to the Hb-content of the red cells, and that the hyperplasia here represents merely a simple physiological increase in functional capacity. He showed also that while the hyperplastic marrow in pernicious anemia is very extensive, occupying as much as 82 per cent. of the cavities of long bones, and is firm and cellular, much of the marrow of secondary anemia is apt to be gelatinous, with comparatively few cells, while the hyperplasia seldom becomes half as extensive as in pernicious anemia.

The writer, from a study of the marrow in cases of grave secondary anemia showing in the blood many megalocytes, observed that the limits of the hyperplasia are seldom very extensive; that the nucleated red cells are usually relatively scarce and of smaller size instead of being overabundant and of very large size as in pernicious anemia. These observations merely corroborate in minor details the original claim of Cohnheim and Rindfleisch.

Yet the essential importance of the changes in the marrow is again brought into question by the reports of cases of pernicious anemia in which the usual alterations in this tissue are wanting.

Six such cases, reported by Laache and Quincke, and one by Geelmuyden, date from too early a period to figure in the present discussion, but Ehrlich[3] in 1888 reported a case of fatal acute anemia in a "bleeder" who suffered principally from metrorrhagia and in whose blood the red cells were reduced to 215,000, the majority of them being undersized, while nucleated red cells were absent, and there was apparently no hyperplasia of the lymphoid marrow. Engel, Lipowski, and Muir[3] have reported similar cases. In 1893 the writer[2] observed an apparently similar condition in a boy, aged eighteen years, of Cuban parentage, who had always appeared pale, and whose sister was extremely pale and moderately anemic. The patient suffered from uncontrollable epistaxis, finally from intestinal hemorrhages, for three weeks before death. During the last week the red cells numbered 456,000, the leucocytes were reduced in number, there were no megaloblasts and no megalocytes, the majority of red cells being oval and undersized. No autopsy was secured, but the anemia appeared clearly to be of idiopathic origin.

Ehrlich, Lazarus, and Engel drew the conclusion from such cases that pernicious anemia is not necessarily associated with hyperplasia of the lymphoid marrow.

The writer has encountered other cases which seem to fall in this class, and for which the term "microcytic type of pernicious anemia" has been suggested, but he has always felt that the grounds for their classification with other acute cases showing megaloblasts and megalocytes are at present entirely inadequate. The reports of Ehrlich do not offer any evidence to prove the relation of these two types of anemia, and it seems entirely premature to draw from such cases any conclusions regarding the pathological anatomy and pathogenesis of true pernicious anemia. In Muir's case the shafts of the bones were thickened, the marrow almost pure fatty tissue, and very few nucleated red cells were found in either blood or marrow.

The extent of the hyperplasia of lymphoid marrow may vary from the involvement of the entire shafts of all long bones to the appearance in smaller foci, only, of the megaloblastic changes. The latter condition is probably seen during the transformation of a grave secondary to a pernicious anemia. Hyperplastic lymphoid marrow may be found in one long bone, while in others the marrow is normal (Lazarus).

Histologically, the marrow in milder cases shows a considerable number of normal red cells, always less than in the circulation, a few normoblasts, and a greater number of megaloblasts sometimes lying in "islands," while in a remarkable case of Rindfleisch's nearly all of the cells in the marrow were large megaloblasts. In most of the writer's cases the neutrophile myelocytes were distinctly deficient, the principal colorless cells being large, hyaline, and mononuclear. The eosinophiles are usually deficient. Evidences of active destruction of blood cells are seen in the presence of macrophages inclosing many red cells, and of many pigmented cells. The megaloblasts may reach an extreme size, 50μ (Rindfleisch); their nuclei are usually eccentric and in various stages of degeneration, while the cell body is polychromatic and very densely staining, or fragmented and pale.

Not only the grade, but the type of lesion in the marrow appears to vary in different cases. The writer has encountered three rather distinct conditions in the marrow of the ribs, vertebra, and femur of fatal cases of idiopathic pernicious anemia.

1. THE COHNHEIM-RINDFLEISCH TYPE (MEGALOBLASTIC DEGENERATION), in which with wide extension of lymphoid marrow an excessive proportion of the cells in the marrow and nearly all the nucleated red cells are megaloblasts or gigantoblasts.

2. EXTREME LYMPHOID HYPERPLASIA (MYELOBLASTIC DEGENERATION), in which the majority of cells are small, hyaline, and mononuclear, while nucleated red cells are scarce, and those remaining are of large size. This type of lesion is identical with that recently described by Naegeli under the term "myeloblastic degeneration."

According to Naegeli, in pernicious anemia the hyperplastic marrow contains 95 per cent. of cells, a little larger than lymphocytes, non-granular, moderately basophile, with reticulated nuclei. These cells he calls *myeloblasts*, and claims that they are the ancestral forms of myelocytes. He finds them in small numbers in the blood of pernicious anemia and secondary anemia, and very abundantly in leukemia. (Regarding the nature of these cells, consult Chapter V.)

3. Recently the writer encountered a case of fatal pernicious anemia of twelve months' duration, without demonstrable origin, in which the marrow showed a remarkable excess of nucleated red cells of *normal size.*

Nature of the Megaloblastic Changes in the Marrow. If Cohnheim's belief is accepted that changes in the marrow in pernicious anemia constitute a reversion of that tissue to the embryonal type in the formation of red cells, pernicious anemia must be regarded as identical with or very closely related to a neoplasm involving the red cells. This view is accepted by Rindfleisch and Ehrlich, but the latter, however, does not apparently press the somewhat strained relation to a tumor process.

The review of our knowledge of the development of the blood, contained in Chapter IV., shows that the cellular processes concerned in the formation of red cells are virtually the same both in adult and in fetal life. In both periods the non-nucleated disks are the result of mitotic division of several series of erythroblasts, each new series containing more and more Hb, until by a process not yet fully understood the non-nucleated disk springs from the last series—*i. e.*, the normoblast. In both adult and embryonal lymphoid marrow the earlier series of nucleated Hb-holding cells appear to be nearly equally represented, while at no period of embryonal life are very large mitotic polychromatophilic cells seen such as abound in the marrow of acute pernicious anemia, and under no other conditions does the marrow contain such an excess of the large megaloblasts as in this disease. The essential difference between the normal formation of red cells in the adult and their defective development in pernicious anemia consists in the failure of the later, more highly specialized series of erythroblasts—*i. e.*, the normoblast. This, of course, is the fundamental nature of a tumor process, in which the highly specialized properly functionating tissues are replaced by excessively cellular, poorly differentiated structures, subject to a great variety of degenerative changes.

Although the gigantoblast of pernicious anemia is never seen in embryonal blood-forming organs, such aberrant forms seem to result from secondary degenerative processes, while it is the absence of normoblasts which is the more important anomaly of blood formation in the disease. The investigations of the thirty years that have intervened since Cohnheim concluded (1868) that pernicious anemia is a reversion to the embryonal type of blood formation seem to have in a considerable degree verified his belief. To what extent pernicious anemia follows the laws known to govern the occurrence and behavior of tumors is an important inquiry which cannot here be further considered.

Pathogenesis of the Disease. Progressive pernicious anemia is believed by some to result through defective hematogenesis, and by others primarily from excessive hematolysis, to which is added *secondarily* defective hematogenesis.

The latter view is based principally upon the abundant evidence of excessive hematolysis, which is undoubtedly the prominent external feature of the disease, upon the reported occurrence of cases of pernicious anemia which were rapidly fatal without yielding evidence of defective hematogenesis in the megaloblastic changes in the marrow, and upon experimental evidence that the prolonged action of certain blood solvents, toluylendiamin, etc., may lead to a condition very similar to progressive pernicious anemia. To some extent this opinion is favored also by the results of repeated losses of blood, which have been shown to lead to a condition closely resembling progressive pernicious anemia both in the blood and in the marrow (Neumann). Moreover, in many cases of grave secondary anemia the blood often approaches in character that of the cryptogenic anemia, and this fact has been accepted as favoring the hematolytic theory, although in cancer of the stomach, which leads to some of the severest grades of secondary anemia, defective blood formation seems quite as likely to figure in the anemia as does excessive hematolysis. The acceptance of this view involves the admission that the changes in the marrow are secondary to the destruction of blood.

The writer has always felt that the correct conception of the disease according to present knowledge is as a process of defective hematogenesis in which megaloblastic degeneration of the marrow is the pathognomonic tissue lesion and excessive hematolysis a constant result.

Hematolysis undoubtedly precedes in many instances defective hematogenesis, but it is a different kind of hematolysis from that which occurs after the megaloblastic changes have become established. The peculiar destruction of blood cells seen in pernicious anemia, marked by excessive deposits of iron, by hemoglobinuria, pathological urobilinuria, by phagocytic englobement of red cells in the marrow, and by peculiar clinical symptoms, appears to be possible only when red cells are defectively formed.

Most convincing demonstration of the propriety of this view was encountered on comparing a case of profound secondary anemia from chronic ulcer of the duodenum with a rapidly fatal case of the cryptogenic disease. In the former case, although the Hb registered only 10 per cent. and the red cells were below 1,000,000, there were only slight changes in the size of the cells, and the patient improved under iron. In the latter case the Hb registered 38 per cent. on first examination, the red cells 1,780,000; but megalocytes and megaloblasts were abundant, and the patient died after an acute illness of four weeks. The essential difference between these cases appears clearly to have been not in the destruction or loss of blood, but in its formation.

It has been amply shown that the system can replace enormous losses if conditions are favorable for the normal production of blood. Lazarus quotes the example of a Russian physician whose health remained little affected by the loss of about four times the entire volume of blood by consecutive pulmonary hemorrhages in the course of a few months. This author also refers to cases of cancer of the stomach in which the anemia remained comparatively harmless until suddenly changing from the secondary type with low Hb-index to the primary with increased Hb-index and appearance of megaloblasts (*Die Anaemie*, p. 43).

The attempted experimental production of pernicious anemia by the use of blood solvents, etc., may or may not have been partly successful, as indi-

cated by Neumann's studies, but their outcome can hardly alter the fact that blood destruction may be continued over long periods without giving the typical picture of pernicious anemia which is at once evident in clinical observation when defective hematogenesis is added to the long-existent hematolysis.

In view of these and many similar considerations it would seem that the belief is justified that it is the megaloblastic degeneration of the lymphoid marrow which constitutes pernicious anemia a separate clinical and pathological entity. Hematolysis may and in some instances does precede, in point of time, the changes in the marrow, as in the pernicious anemia following malarial cachexia; but this destructive influence at once takes on a new character and produces specific results when acting on defectively formed cells, as is shown by the comparison of the iron-content of the liver in secondary and in progressive pernicious anemia.

The occurrence of rapidly fatal cases of the disease and the transformation of a grave secondary anemia (cancer of pylorus) into true pernicious anemia may be explained by a rapid and general or slow and partial establishment of the marrow changes. In the former case the changes in the blood will be pronounced from the first; in the latter it may be difficult to determine the exact point when a grave secondary becomes a true pernicious anemia.

Changes in the Blood.

Chemistry. The specific gravity of the blood is constantly reduced, owing to the loss of both the albumins of the plasma and the Hb of the red cells. The gravity is here a much less reliable indication of the Hb-content than in chlorosis, in which the Hb alone is markedly affected (Hammarschlag, Dieballa). Nevertheless, it is just in this disease that the information furnished by the specific gravity is of peculiar interest, as it concerns those principles in the plasma of which little can be learned by other, simple clinical tests.

Grawitz finds that the specific gravity of the blood may fall below 1.030, or below that of normal serum. Dieballa had two cases showing a gravity of 1.028, with red cells 404,000 and 500,000; while Copeman, using Roy's method, reported a case with a gravity of 1.027. That there is no close relationship between the numbers of red cells and the specific gravity appears in Dieballa's tables, in which a gravity of 1.038 was obtained in one case showing 784,000 cells and in another with 1,400,000 cells. The total albumins of the blood are much reduced, but the loss affects principally the red cells. Grawitz finds the loss of albumins of the serum to be distinctly greater in secondary anemia than in progressive pernicious anemia.

The great reduction in total albumin of the blood is indicated by the low percentage of dry residue found by v. Jaksch, Grawitz, and Stintzing and Gumprecht, who in very severe cases obtained between 9 and 12 per cent. (normal 20 to 24 per cent.), while the red cells numbered between 534,000 and 1,700,000. That the loss of albumin is due principally to the changes in the red cells appears from the fact that the specific gravity and dry residue of the serum are but slightly altered even in severe cases

Thus Grawitz found a gravity of the serum of 1.024 (normal, 1.027 to 1.029), dry residue 7.08 per cent. (normal, 10 to 10.5 per cent.), while the gravity of the blood fell to 1.036, and its dry residue to 11.45 per cent. (normal, 21 to 24 per cent.). While the albumins of the blood were reduced, therefore, one-half, those of the serum fell only one-fifth. Similar results were obtained by Dieballa. This loss of albumins of the whole blood must be referred to the loss of fibrinogen and to the diminution of red cells. v. Jaksch's analyses[2] of red cells in pernicious anemia led him to conclude that those remaining are abnormally rich in albumins—a view which may seem to accord with the large size of these cells. By estimating the N of 100 grs. of centrifuged red cells he obtained 6.48 grms. N, 40.5 grms. albumin (normal 34.5). Grawitz's analyses, however, do not support this conclusion.

The relatively high proportion of albumin in the serum in pernicious anemia distinguishes this disease, as Grawitz has shown, from many forms of secondary anemia arising from hemorrhage, malignant tumors, etc., with which the albumins of the serum are much reduced.

The *resistance* of the red cells has not apparently been tested, although Limbeck (p. 163) found their isotonc tension somewhat increased (resistance diminished) in two cases of grave anemia.

Copeman noted that the hemoglobin crystallized readily from the blood of a case of pernicious anemia, while it is very difficult to obtain such crystals from normal human blood.

Morphological Changes. THE WHOLE BLOOD is much reduced in quantity, as is shown by the scanty content of vessels and viscera at autopsy and by the imperfect filling of the superficial vessels during life. This reduction in bulk is often so marked as not to require for its demonstration any attempts at accurate measurement.

Smith's conclusion that the volume of blood in pernicious anemia is increased strongly suggests that in this disease his method of determination by the oxygen capacity is unreliable.

COAGULATION is very feeble, so that on standing it was early noted (Ponfick) that the red cells settle to the bottom, the leucocytes form a middle layer of variable depth, while the plasma deposits nearly all its cells without interference from clotting. To the feeble clotting is probably referable the very slow separation of serum from the clot, as observed by Lenoble.

The COLOR of the blood may in mild cases be almost normal; it is usually very pale; while in extreme cases visible currents of colorless serum mingle with the drop on very slight pressure. On this account a liberal puncture may be required to obtain a drop suitable for examination. A very dark color has been observed by Furbringer and, after transfusion, by Gusserow.

Hemoglobin. The percentage of Hb varies from a moderate reduction (70 per cent.) to such a low point that accurate estimates are impossible, while the Fleischl instrument indicates less than 10 per cent. In the average case the Hb registers between 20 and 40 per cent. A rather high percentage of Hb with severe clinical symptoms and pronounced morphological cha[illegible] in the red cells is not infrequently seen. Lazarus refers to a [illegible]ng 65 to 70 per cent., but otherwise with pronounced symptoms. The Hb is usually not so low as in secondary anemias of equal severity.

A RELATIVELY HIGH HB-INDEX is one of the characteristic features of the blood. In many cases, especially when the megalocytes are very abundant, the Hb-index is *above normal* (1 to 1.75), and in such cases the index is apt to rise as the anemia increases, and to fall during intervals of improvement. In a larger group of cases the index does not vary greatly from the normal, when the excess of Hb in megalocytes balances the loss in other cells. In other cases the index is for long periods below normal, but still relatively high as compared with secondary anemia and chlorosis. Thus the Hb-index in chlorosis *averages* about 0.50 (Cabot, Bramwell), but in pernicious anemia the index *seldom approaches* that figure. The low Hb-index is usually seen in chronic cases, while the highest indices obtained by the writer were in more rapid cases especially just before death.

In some cases of secondary anemia the Hb-index may approach the normal, so that comparatively little diagnostic importance can attach to the lesser grades of increase in Hb.

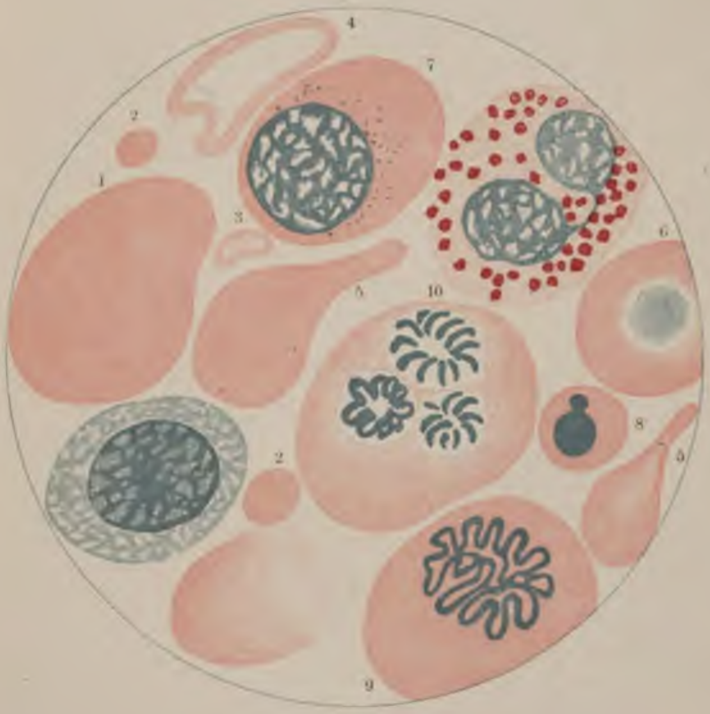
Red Cells. In the average case of established pernicious anemia the red cells vary slightly above or below 1,000,000. Well-marked morphological changes may exist, however, when the red cells number over 2,000,000; but earlier stages of the disease have so far escaped notice, indicating that pre-existing anemia is an essential predisposing condition.

Although Quincke reported 143,000 red cells in a case which recovered and Hayem 292,500 in a fatal case, more recent reliable statistics show that life is seldom prolonged when the cells fall below 400,000. Even this extremely low proportion is below the count in many fatal cases, as many patients succumb when the red cells are about 1,000,000. Most fatal cases show at death between 300,000 and 600,000 red cells. The reduction in cells, in cases under treatment, seldom progresses uniformly, intervals of improvement, followed by rapid relapses, continuing until the end.

Changes in the *form*, *size*, and *staining reaction* of the cells are even more important than their reduction in number.

While the largest cells of normal blood measure about 9μ across, in pernicious anemia the increased size of the cells is characteristic of the disease, the average diameter being estimated by Lazarus at 11μ to 13μ, while some may reach a diameter of 16μ to 18μ (Askanazy). Cases vary greatly in regard to the extent and uniformity of this lesion. In one of the writer's cases, not of unusual gravity, at least 90 per cent. of the cells measured from 11μ to 16μ. Megaloblasts were very numerous, and microcytes extremely rare. *It may be said that unless 33 per cent. of the cells are distinctly oversized the diagnosis of pernicious anemia should be made with reserve.* Usually, but not always, the proportion of megalocytes accords with the severity of the condition. Lazarus found in eight severe cases 56 to 71 per cent. of megalocytes, in five cases during periods of improvement 33 to 50 per cent., or during complete remissions 0 to 14 per cent.

PLATE VI.



Progressive Pernicious Anemia. (Eosin and Methylene Blue.)

Fig. 1. Megalocyte with excess of Hb.
Figs. 2. Microcyte with excess of Hb.
Fig. 3. Microcyte deficient in Hb.
Fig. 4. Poikilocyte deficient in Hb.
Figs. 5. Poikilocyte with excess of Hb.
Fig. 6. Polychromasia of Maragliano.
Fig. 7. Megaloblast, resting vesicular nucleus, basophilic granules in cytoplasm.
Fig. 8. Microblast.
Fig. 9. Megaloblast in mitosis. Thread stage.
Fig. 10. Megaloblast. Pathological mitosis.

The Hb-content of these megalocytes is in typical cases distinctly increased and the cell is flat and uniformly opaque instead of showing the biconcave discoid shape. The writer has found this character of the megalocytes a very reliable differential sign between true pernicious anemia and the grave secondary anemia of cancer, etc. In the latter conditions the great majority of megalocytes are usually deficient in Hb. (Cf. Plates VI. and VII.)

MICROCYTES are a somewhat less characteristic cell-form in pernicious anemia. They may be only slightly under normal size or be found in severe cases as mere points of reddish staining protoplasm not over 1μ in diameter. They may contain an excess or deficiency of Hb. In the early stages of the disease the writer has found them less frequent, but in moribund cases they rarely fail to appear in considerable numbers. The division of forms between megalocytes and microcytes is then often complete, no normal cells remaining.

DEFORMED RED CELLS (POIKILOCYTES) of all sizes are commonly present in considerable numbers, probably resulting from the ameboid properties of the cells and frequently from traumatism. The oval shape of many cells both large and small was early noted by Osler and Gardner, but this type of cell is much more characteristic of *purpura hæmorrhagica.* Pear-shaped cells are perhaps the most common form, the narrow point representing a pseudopodium. Cells very deficient in Hb are apt to bend into horseshoe or pessary shapes. Cells rich in Hb less often become deformed.

AMEBOID MOTION of red cells in pernicious anemia may be observed in fresh preparations. The moving cells usually content themselves with the extrusion of pseudopodia, and with changes in shape which Muir and Gulland believe are not true ameboid phenomena, but the writer has seen active locomotion of lenticular microcytes.

NUCLEATED RED CELLS may be said to occur in all cases of pernicious anemia, but their demonstration sometimes requires prolonged search. It has been shown by Ehrlich and verified by nearly all later observers that *megaloblasts* constitute the majority of such cells in pernicious anemia, and there is at present no ground on which to deny their pathognomonic significance, when present in large numbers. In typical cases of the disease they are almost or quite exclusively present, while normoblasts are scarce. Their numbers seem to depend largely on disturbances of the circulation and other accidents; but Ehrlich claims that the extent of the megaloblastic degeneration of the marrow may be determined by the numbers of these cells in the blood. This rule probably holds in general, but in the writer's experience there have been exceptions. In other cases both normoblasts and megaloblasts appear in the blood, in which case it is found that the megaloblasts outnumber the normoblasts, indicating that the defective formation of cells has become uppermost.

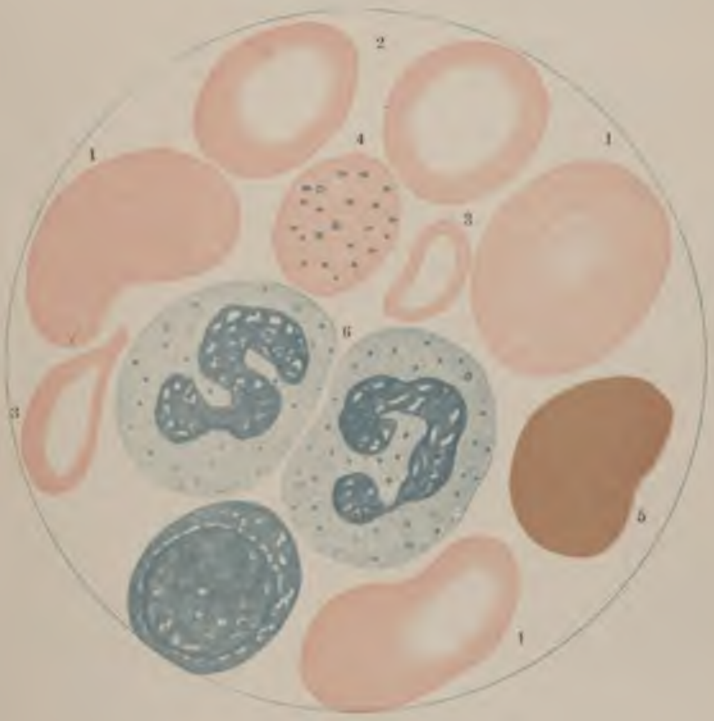
From the considerations mentioned under the etiology and pathogenesis of the disease, it will be seen that the crucial point in the diagnosis between secondary and pernicious anemia is encountered in practice, in the proportions of normoblasts and megaloblasts to be found in the blood. If, as appears to be fully attested, a grave secondary may be transformed into pernicious anemia, the change must consist primarily in a megaloblastic degeneration in some portion of the marrow. The normoblasts of the circulation must then be gradually replaced by megaloblasts.

These theoretical deductions appear to be borne out in clinical experience since the appearance of megaloblasts accompanies a more severe type of anemia. Lazarus finds normoblasts rarely associated with megaloblasts, and this has been the writer's experience, except in children, in whom both are frequently found together. Schaumann saw both forms in all his cases of *bothriocephalus*. Cabot noted that megaloblasts and normoblasts are frequently associated in pernicious anemia and that the former increase when the disease grows worse. Askanazy found many megaloblasts in a case of *bothriocephalus*, while after expulsion of the worms they were steadily replaced by normoblasts. Dorn observed an idiopathic case promptly improve after the sudden appearance of many normoblasts. From Coles' and Solley's observations it is seen that there may be very marked variations in the numbers and to a less extent in the proportions of normoblasts and megaloblasts in the blood, and that in the early stages of the disease normoblasts may predominate. On both clinical and pathological grounds, therefore, it is justified to class an anemia as pernicious when the megaloblasts outnumber the normoblasts, or when any considerable proportion (33 per cent.) of the red cells are megalocytes with increased Hb. The writer has encountered clinically and at autopsy cases of cryptogenic pernicious anemia in which very large numbers of erythroblasts, of normoblastic or slightly larger size, were present in the blood and the marrow.

A question frequently arises regarding the value of a prolonged search for megaloblasts in order to establish the diagnosis of pernicious anemia, since if only one nucleated cell be found and that a megaloblast, the chances favor the presence of an excess of such cells over normoblasts. It is quite possible, however, that the first few nucleated cells should be of large size, while the next three or four prove to be normoblasts, and it appears much more rational to base the diagnosis upon the general morphology of the blood and on all clinical data, than to rely upon any single isolated sign. Nevertheless, the discovery of a single very large megaloblast with abnormal nucleus is virtually a pathognomonic sign of pernicious anemia. Especially significant is the discovery of megaloblasts in which the nucleus constitutes four-fifths of the cell, and is reticular in structure instead of compact, and in which the cytoplasm is strongly polychromatic (Engel's metrocyte).

The numbers of nucleated red cells in pernicious anemia vary greatly. Frequently they are scarce and their demonstration requires patient searching. Or, of two slides taken at the same time one may contain several and the other apparently none. Usually they increase with the severity of the blood changes. The occurrence of normoblastic blood crises has been referred to as a favorable sign (Dorn). Coles found over 6000 nucleated red cells per c.mm., the majority of large size, in a fatal case, and Solley found 35,000, mostly normoblasts, shortly before marked improvement in the blood of a case of ascariasis with pernicious anemia. Straus and Rohnstein followed a case in which only normoblasts were present until shortly before death, when very large numbers of megaloblasts suddenly appeared

PLATE VII.



Secondary Pernicious Anemia. (Eosin and Methylene Blue.)

Figs. 1. Megalocyte.
Fig. 2. Red cells of normal size deficient in Hb.
Fig. 3. Microcyte deficient in Hb.
Fig. 4. Red cell showing granular degeneration. (Punctate basophilia.)
Fig. 5. Megalocyte with excess of Hb. (Polychromasia of Gabritschewsky.)
Fig. 6. Polynuclear leucocyte. Nodal points of cytoreticulum.

in the blood. The writer has found them unusually abundant in a case in which nearly all the red cells were very large, and has seen them nearly as numerous as the leucocytes during antemortem leucocytosis. Distinct microblasts are comparatively rare.

The occurrence of *mitotic nuclei* in megaloblasts, first described by Luzet, is usually limited to the severer stages of the disease, in which their presence is pathognomonic of the condition. Schaumann, however, found them in a case that recovered. The presence of three or more *unequal asters* in one gigantoblast, as occasionally found in severe cases, is one of the most significant pathological signs within the range of blood analysis. (Plate VI., Fig. 10.)

DEGENERATIVE CHANGES IN THE RED CELLS not only cause the appearance of abnormal cell forms, but lead to a series of *chemical and morphological alterations* plainly demonstrable in stained specimens. *Polychromatophilia*, referring to the development of a brownish staining quality of the Hb, is constantly present, usually more marked in the severer cases It appears probable that the change is in some cells preliminary to the solution of Hb in the plasma, but, according to Engel, it indicates very rapid formation of cells and incomplete metamorphosis of nucleus. It is most marked and nearly constantly present in megaloblasts, some of which stain diffusely brownish blue.

Hemoglobinemia is constantly present in the severer cases and is indicated by a distinct brownish red staining quality of the dried plasma.

PUNCTATE BASOPHILIA (granular degeneration, Grawitz[3]) of red cells is of moderately frequent occurrence, both in megalocytes and especially in megaloblasts. As previously shown, this abnormality is sometimes referable in the megaloblasts of pernicious anemia to karyorrhexis of degenerating nuclei. In other cells the blue granules are probably reappearing particles of the nuclear remnant of ordinary red cells (Maximow).

SCHISTOCYTOSIS, or the separation of fragments of red cells, is, according to Ehrlich, the mode of origin of many microcytes in pernicious anemia.

HYDREMIA of red cells probably causes many smaller cells to appear much larger than normal It has already been shown that hydremia of the plasma is probably responsible for the increased diameter of many red cells, but in pernicious anemia the majority of megalocytes, especially those with increased Hb, are undoubtedly derived from megaloblasts.

ABSENCE OF ROULEAUX is a constant and very characteristic feature of the disease, and results not only from the reduction in the number of cells, but principally from chemical changes in their membranes and protoplasm.

The Leucocytes. In well-established cases the leucocytes are markedly reduced in number. This condition is clearly referable to the megaloblastic lesion in the marrow, which leads to an excessive proliferation of megaloblasts at the expense of other marrow cells (Rindfleisch). Progressive hypoleucocytosis is the rule, the white cells falling very low in extreme cases (1500 to 2000, Hayem). Of the colorless cells which remain, a diminished proportion are neutrophile, the majority being large and small basophilic cells. With the reduction in total numbers there is a relative lymphocytosis, very slight in the mild cases and very marked (85 per cent., Da Costa; 90 per cent., Geissler) in severe stages. The *eosinophile cells* usually vary within low normal limits. In the severer cases they are apt to be reduced (Grawitz), and rarely may be absent (Cabot); but, contrary to Neusser's belief, they are very scarce in mild and present in some fatal cases. In the marrow, the writer has found them usually much reduced in number. *Myelocytes* are usually present in small numbers, Cabot finding them in forty-two of fifty-two cases, and Da Costa in twenty-nine of thirty-one cases. The writer finds these cells in pernicious anemia almost invariably of the smaller type, but has seen isolated specimens of the larger (Cornil's) neutrophile cell, which is abundant in leukemia.

Mast-cells were increased in some of the writer's marrow smears, but he has never seen more than single examples in the blood smear.

Hyperleucocytosis in pernicious anemia occurs as the result of complications, as an antemortem phenomenon, rarely without discoverable cause in course of the disease, and in one case (Dorn) it appeared to be associated with a normoblastic blood crisis, and heralded a favorable turn in the disease. In many of these cases the leucocytosis is very marked and so many myelocytes or lymphocytes are present as to lead to the diagnosis of acute leukemia. Thus Geissler found 90 per cent. of lymphocytes among 34,000 cells in one such case, and Grawitz[4] reports 20 per cent. of myelocytes among 55,000 white cells in another, while Freund counted 25 per cent. of these cells in a chronic case and Arneth 14 per cent. in an acute case. The occurrence of such changes in the blood of pernicious anemia should be carefully considered when a diagnosis of acute leukemia is suggested.

There have recently appeared rather numerous reports of acute lymphatic or myelogenous leukemia without leukemic lesions in the viscera. An examination of several of these reports shows that the writers have been dealing with cases similar to those which Grawitz and others described as pernicious anemia with leucocytosis and high proportions of myelocytes or lymphocytes. There can be little doubt that Grawitz's interpretation of these cases is the correct one, and that without specific lesions in the viscera leukemia cannot exist.

RESUME OF THE CHIEF FACTS CONCERNING PROGRESSIVE PERNICIOUS ANEMIA.

Definition. The term should be applied to all forms of grave anemia showing megaloblastic or myeloblastic degeneration of the bone-marrow, a lesion which is usually accompanied by an excess of megaloblasts over normoblasts in the blood, or at least 33 per cent. of megalocytes (or less during remissions).

Etiology. The largest group of cases remains of cryptogenic origin, but is possibly to be referred to intestinal intoxication. Various lesions of the gastro-intestinal tract are underlying causes, including chronic gastritis with or without stenosis of pylorus, and small carcinomata of pylorus.

Of intestinal parasites, *Bothriocephalus latus* frequently, and other parasites occasionally, lead to the disease.

Repeated hemorrhages in rare instances cause typical pernicious anemia. *Pregnancy* has no specific influence, but the cases which follow parturition are probably referable to hemorrhage and various unhygienic conditions.

The disease is referable to *pernicious malaria* often; to *syphilis, prolonged diarrhea, bad hygienic conditions*, probably; to typhoid fever, yellow fever, and other infectious diseases, doubtfully; but not to functional or organic disturbances of the nervous system.

Lymphosarcoma of the marrow was present in one reported case.

Pathological Anatomy. The essential lesion of the disease is a megaloblastic metaplasia of the lymphoid marrow which is invariably present, is pathognomonic of the disease, and in many respects resembles a tumor formation affecting the progenitors of the red cells.

Pathogenesis. The disease does not exist until defectively formed red cells are being destroyed in large numbers by some hemolytic agent.

Changes in the Blood. The red cells may be reduced to about 200,000, but usually number about 1,000,000. Megalocytes with increased Hb constitute from 33 per cent. to 90 per cent. of the cells, but during remissions they may be very scanty.

Megaloblasts are very scarce, or very numerous, and are either exclusively present or outnumber the normoblasts. During remissions normoblasts may be the more numerous. Microblasts are rare.

The Hb-index varies, but in the advancing stages it is usually above normal, or during periods of improvement or remission may be below normal. If below normal, it is usually higher than in any other form of anemia.

In the fresh blood coagulation is very feeble, rouleaux do not form, there are doubtful evidences of ameboid motion of poikilocytes, but occasionally active locomotion of microcytes. Hemoglobinemia often exists, but is sometimes, possibly, an artifact.

Degenerative changes in red cells are extensive, including poikilocytosis, schistocytosis, diminished resistance and solution of red cells, polychromasia(Gabritschewsky), punctate basophilia,and karyorrhexis in megalocytes and megaloblasts

Leucocytes are usually reduced in proportion to the severity of the lesion in the marrow and the progress of the disease, and relative lymphocytosis is the rule. Leucocytosis arises usually from complications, rarely with "blood crises," and often just before death. Eosinophiles are commonly deficient and a few medium-sized myelocytes can usually be found. Either lymphocytes or myelocytes may be found in such proportions as to suggest leukemia.

Chemistry. The blood is much reduced in bulk. The specific gravity has no constant relation to the number of red cells or proportion of Hb, but follows the loss of albumins, of cells, and of plasma. It may fall as low as 1.027. The albumins and specific gravity of the serum are much less reduced than in grave secondary anemias. The resistance of the red cells is reduced and Hb may crystallize readily. The hydremia of the plasma affects the red cells and is probably responsible for some of their increase in size.

The diagnosis *may* rest upon the presence of numerous megaloblasts and megalocytes with increased Hb; 33 per cent. of megalocytes with increased Hb; an excess of megaloblasts over normoblasts; a single gigantoblast or megaloblast in pathological mitosis.

The diagnosis *cannot* rest on an extreme reduction of red cells.

The diagnosis *may require* the complete summation of all clinical and morphological data, as well as observation on the course of the disease, or even the microscopic examination of the marrow.

The cases of fatal idiopathic anemia of microcytic type (Ehrlich, Engel, and others) cannot at present be classed with the Addison-Biermer progressive pernicious anemia.

The prognosis is very difficult to determine.

The most severe case on record recovered (Quincke's with 143,000 (?) red cells). Severe cases of cryptogenic origin may recover. The most unfavorable are the very acute idiopathic cases, and those of slower development from gastro-intestinal lesions.

The sudden development of well-marked changes in the blood commonly indicates a severe course, but such cases have recovered. The slow relapsing cases, with gradual changes in the blood and absence of megaloblasts usually die from their anemia.

A high Hb-index goes with well-marked changes in the blood and marrow and is of unfavorable import. Lower Hb-indices are seen in chronic cases and in remissions of more acute cases.

A reduction to 600,000 to 700,000 red cells in this disease is seldom survived. Marked degenerative changes in megalocytes and megaloblasts and marked hemoglobinemia go with the severe cases.

Extreme reduction of neutrophile and eosinophile leucocytes is an unfavorable sign; while leucocytosis commonly precedes death by a few days.

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CHAPTER VIII.

LEUKEMIA.

Historical. Probable cases of leukemia were observed and commented upon from the beginning of the nineteenth century by Bichat (1801), Andral (1823), Hodgkin (1832), Donne (1830), and many others, and the disease was imperfectly recognized in current textbooks (Piorry, Velpeau, Rokitansky), in which it was regarded as an obscure suppurative "hematitis" (Piorry). Donne, however, in 1839, found in his case at autopsy that the blood cells were more than one-half "white or mucous globules," and attributed the disease to a failure of transformation of the leucocytes into red cells.

The first step in the elucidation of the disease was made when Craigie, in 1841, demonstrated an entire absence of suppurative foci in the body and concluded that the purulent material was absorbed from the enlarged spleen, of which the histologial structure "does not favor the gathering of pus in abscesses."

In the same issue of the *Edinburgh Medical Journal* Bennett described a second case, demonstrating an entire absence of suppurative foci, describing accurately the gross appearance of the blood in bulk, and under the microscope recognizing the leucocytes as identical in appearance with pus cells, and adding to the lesion in the spleen a uniform enlargement of lymph nodes. Regarding the nature of the disease he concluded that the excess of leucocytes in the blood must be classed as pus; that in this case the pus was formed, entirely from the action of a zymotic principle in the blood, quite apart from inflammatory processes; and that the enlargement of the liver and spleen and the pyoid condition of the blood "lead us to conjecture that in some way they stand in the relation of cause and effect."

Virchow, a few weeks later, described a case complicated by superficial abscesses, and termed the condition "*weisses blut*" or leukemia. In a series of later articles he distinguished more clearly than Bennett had done between pyemia and leukemia, and emphasized the relation between the changes in the blood and the lesions in the blood-forming organs, holding the disease to be a primary affection of these organs.

In 1851 Bennett contributed the first monograph on the disease, collecting thirty-seven cases, describing and depicting the microscopic appearance of the blood, reporting the first chemical analyses, giving very reliable descriptions of the symptomatology and pathological anatomy, reporting cases of idiopathic splenic tumor without leukemia, and employing the term *leucocythemia* as preferable to leukemia.

In 1847 Virchow observed a case in which the spleen was very slightly and the lymph nodes greatly enlarged, and in his review of the subject in 1853 he pointed out the grounds for division of the disease into splenic and lymphatic varieties. The chief of these grounds was the presence in the blood of the above case of cells which resembled those of the lymph nodes.

The leucocytosis was first demonstrated during life by Fuller (1846), who also believed that the alterations in the blood were not of inflammatory origin. In 1851 Vogel also demonstrated the leucocytosis during life and attempted to count the cells.

In 1869 Neumann[3] described the lesions of the marrow, demonstrating the origin of the myelocytic variety of the disease, and his observations were verified and extended by Waldeyer (1871). Ponfick noted that in some cases the marrow is light yellow and puriform, while in others it retains more or less fleshy color, a variation which he referred to the extent of the cellular hyperplasia. The hyperplasia of the gastro-intestinal lymphatic structures and metastatic tumors of the pleura were described by Friedrich in 1857, while the enlargement of the liver was referred to collections of cells probably derived from the blood, by Bottger, in 1858.

In the morphology of the blood it was shown by Bennett and Virchow that the leucocytes of the blood were identical in appearance with those of pus, although Virchow was careful to point out that they were not therefore of similar significance These early observers both described the cells as mononuclear or polynuclear, or devoid of nucleus (Virchow) and granular or hyaline. Virchow and most others mistook the eosinophile granules for fat particles. In his case of lymphatic leukemia Virchow describes the cells of the blood as resembling those of the lymph nodes.

No important advance in the knowledge of the minute structure of leukemic blood cells was made until Schultz and Erb, in 1865, classified leucocytes according to the character of their protoplasm. Neumann's discovery of the myelocytic type of the disease also directed more careful attention to the characters of the new leucocytes and strengthened the suspicion that the new cells of leukemic blood were not all of the same varieties as seen in normal blood. The question was finally settled by Mosler,[2] who punctured the sternum during life and secured from the marrow large numbers of the identical cells seen in the peripheral circulation. It was at once concluded that these cells must be characteristic of the myelogenous type of the disease.

In 1878 Neumann contributed an important study claiming that some cases are of purely myelogenous origin, that in no case is the marrow uninvolved, and that a lesion of the spleen or lymph nodes alone is incapable of producing the disease. The most recent studies of the disease have tended to support this belief, that leukemia of both types is always of myelogenous origin (Walz, Pappenheim, Hirschlaff, and Kormoczi).

In 1879 and 1880 Ehrlich's studies added greatly to the knowledge of leukemic changes in the blood, and were especially important in permitting greater accuracy in diagnosis. It, then, for the first time, became possible to distinguish all cases of lymphatic from myelocytic leukemia and to recognize early stages and other obscure phases of the latter type of the disease. The first pure case of myelocytic leukemia was described by Litten.[1]

In the diagnosis of myelocytic leukemia Ehrlich was able to find in the blood three chief signs: (1) mononuclear cells with neutrophile granules, (2) an increased number of eosinophile cells, and (3) normoblasts or, later, megaloblasts.

His conclusion that an increase of eosinophile cells, first noted by Jaderholm and Schwarze, is pathognomonic of the disease was soon relinquished. As shown by Müller and Rieder, it is not so much the increased numbers, but the peculiar forms of eosinophile cells which are characteristic of leukemia, although their numerical excess is often of itself pathognomonic. The neutrophile myelocytes, as long before claimed by Mosler, were therefore again accepted as the most important cell form. Through the contributions of Mosler,[2] Ehrlich, Cornil, Müller,[1] Limbeck, Rieder, Troje, and many others, it was shown that these cells are derivatives of the marrow and probably peculiar to the blood of leukemia. The diagnostic importance of Cornil's large myelocytes with pale eccentric nucleus was established through the observations of Eberth, Eisenlohr, Mosler, Litten, Hayem, and especially of H. F. Müller.

Later it came to be recognized that neutrophile myelocytes are occasionally seen in small numbers in many other conditions. Consequently there remained, as a diagnostic feature of the blood, only the presence of a *large proportion* of myelocytes, and it was seen that the genuineness of some previously reported cases was open to doubt (*e. g.*, Litten's).

Diminution or absence of ameboid motion in the myelocytes of leukemia was described by Biesiadecki, Löwit,[1] Hayem, Müller, Gilbert, Rieder, and others. That this loss of ameboid motion is associated with a loss of reproductive capacity was stated by Mayet.[1] A partial loss of ameboid activity of other leucocytes was observed by Neumann and Cavafy, while J. Weiss does not admit any difference in ameboid properties of the white cells of leukemia and of other leucocytes. Jolly depicts slow ameboid movements of both neutrophile and eosinophile myelocytes in leukemic blood at 38° C.

The discovery of *nucleated red cells* was followed by their demonstration in the blood of leukemic cadavers by Erb, Bottger,[2] and Klebs,[1] and in the circulating blood by Neumann.[5] The comparative absence of these cells in lymphemia was first noted by Hayem and verified by Wertheim, Rieder, and Dock. Megaloblasts were first distinguished in leukemia by Ehrlich's pupil, Utheman, and by Troje.

Likewise the discovery of mitotic division of leucocytes by Flemming and Arnold led, after a time, to the demonstration of mitotic figures in the white cells of both the viscera and the blood of leukemia (Arnold, 1884; Hayem, 1889; H. F. Müller, 1889).

Acute leukemia was first described by Friedrich in 1857 in a case lasting six weeks. Other reports of similar cases were added slowly, so that in 1889 Ebstein collected sixteen cases lasting from five to twenty weeks, many of which appear to have been of the lymphatic variety.

The knowledge of the *chemistry* of the blood has kept pace with that of the morphology. The first chemical analyses were those of Robertson, 1850 (January), and of Parkes, while a few months later Strecker examined the blood of a case of Vogel's. Later important contributions were made by Becquerel, Robin, and Scherer. In these early analyses the chief chemical alterations in the blood were fully indicated. The specific gravity of the blood was found as low as 1.036 to 1.019, that of the serum 1.023 to 1.029. The iron was shown to be markedly diminished and the proportion of water increased, with a loss of albumins, especially of the red cells. Scherer reported the isolation of several organic acids in considerable traces, and of hypoxanthin, a body previously isolated from the spleen, while the presence of increased uric acid was demonstrated by both Scherer and Parkes, and later by Fowlwarckzny, Koerner, and Mosler. The later chemical analyses of leukemic blood and tissue have been contributed by Stintzing and Gumprecht, Limbeck,[2] v. Jaksch, Salkowski, Bockendahl and Landwehr, Freund and Obermayer.

Etiology. Age. The histories of the earliest cases (Bennett) indicated that the disease may occur at almost any period of life. Later statistics showed that in the male sex the liability increases from childhood to the thirtieth or fortieth years, thereafter declining. In women most cases occur during active sexual life. In infants cases have been noted from birth, but the few cases recorded in childhood indicate that this period is comparatively immune. Audeod observed the disease in fifty-six children; in the first year, eleven times; two to four years, twelve times; five to nine years, twelve times; ten to fifteen years, twenty-one times; the majority were boys. Of 135 cases collected by Ehrlich, Grawitz, Litten, 87 were males, 48 females.

Heredity has figured in some remarkable cases, but its influence cannot usually be traced. Sanger observed a leukemic infant, born of a mother who was healthy and remained so. On the other hand, many leukemic women have given birth to healthy children. The influence of direct heredity is rare. Casati cited the case of a girl, aged ten years, whose father and grandmother were leukemic, while Cameron observed a case in a woman, whose grandmother, mother, brother, and two children were leukemic. Collateral heredity, the affection of brothers and sisters, has been frequently observed (Naunyn, Duret and Vaquez, Senator, Ortner, Casali, Greene, Eichhorst, Bernier).

Trauma has been demonstrated to be a direct exciting cause of leukemia in a large number of authentic cases The traumatism has been applied directly to the spleen (Velpeau, Wallace, Mosler, Grawitz, Ebstein), or to the bones (Virchow, Grawitz, Mursick), in which case the myelogenous type commonly develops. The traumatism may consist in general concussion. Pre-existing leukemia was thus rendered rapidly fatal in a case of Greime.

The significance of the alleged cases of leukemia following *splenectomy* will be considered under that topic.

Intestinal intoxication has been urged as the exciting cause of leukemia by Vehsemeyer, who believes that the failure of conversion of peptone into harmless albumins of the blood is the important causal factor in the disease. This theory was suggested by the frequency of gastro-intestinal disturbances and lesions in leukemia, and by some observations of Kottnitz, which indicated that in leukemia the gastro-intestinal mucosa loses to some extent the power to transform peptones.

The effects of **bad hygiene** have been traced in a large number of cases (Mosler), but no social position or occupation distinctly predisposes to the disease.

Pregnancy appeared to be the predisposing or exciting condition in acute cases reported by Lauenberg, Stewart, Askanazy, Thompson and Ewing.

Other diseases predisposing to or directly exciting leukemia have been reported as follows:

Syphilis, acquired and constitutional, especially in children, and exciting usually the myelogenous type; by Steinbrugge, Frankel, Mosler.

Malaria, either acute or chronic (Müller,[2] Gowers, Osler), 33 per cent. Of 150 cases collected by Gowers, a history of previous malaria was obtained in 39, while in many the spleen had remained enlarged after the malarial attack. The disease has been attributed to diphtheria by Orth, and to typhoid fever, smallpox, and pneumonia by Gowers.

Influenza has several times been reported as leading to acute or chronic leukemia (Hinterberger, Frankel,[1] Litten[1]).

Rachitis, tuberculosis, and hypertrophic intestinal lymph follicles from chronic catarrh (*constitutio lymphatica?*) have been noted, singly or combined, in many cases in children (Mosler[1]). In a series of cases, both of myelemia and of lymphemia, extensive lesions of tuberculosis have been found.

Stomatitis, ulceration of gums or tonsils, preceding or early accompanying leukemia, have been reported by many observers (Ebstein, Hinterberger), but are probably secondary lesions.

The transformation of pernicious anemia into leukemia has been reported in several cases (Waldstein, Litten,[1] Masius and Francotte, Leube and Fleischer, Musser). It does not appear, however, that any of these cases indicate a relation between progressive pernicious anemia and leukemia. They seem to the writer to be cases of leukemia in which there was a temporary reduction in leucocytes at the time of first observation, or instances of marked terminal leucocytosis. The case of Masius and Francotte, in a patient suffering from ankylostomiasis indicates that severe secondary anemia may predispose to leukemia. An apparently genuine instance of transformation of pseudoleukemia into acute leukemia is reported by Mosler,[3] and Troje has maintained that pseudoleukemia is pathologically a forerunner of leukemia. This is a view of a large portion of the French school, but recent reports of such cases furnish, as a rule, unsatisfactory evidence that such transformations actually occur (cf. Wende, and Rosenfeld).

The genuineness of many reported cases of leukemia following infectious diseases, splenectomy, childbirth, anemia, etc., has been seriously doubted, and it has been agreed on all hands that spurious cases have crept into the literature, especially before the general employment of Ehrlich's methods and criteria. The recent demonstration of numerous myelocytes or lymphocytes in inflammatory leucocytosis (Engel, the writer, etc.), the extreme leucocytosis (1 to 2), observed by Schede and Stahl in osteomyelitis, and Musser's observation of a proportion of one white to four red cells in pernicious anemia, render it obvious that the diagnosis of acute leukemia in the absence of microscopic examination of the marrow is often a hazardous undertaking.

Infectious Origin. The chief ground for a belief in the infectious nature of leukemia is found in the rapidly fatal course of some acute cases and their striking clinical resemblance to other undoubted infectious diseases. Actual contagiousness is suggested by the report by Obrastzow, of an acute case developing in a hospital attendant six weeks after nursing another fatal acute case of the myelocytic type.

Hintze and Frankel argue in favor of an infectious origin, and Askanazy, finding no leukemic lesions in a dead fetus from a leukemic mother, concluded that the disease is not excited by a circulating principle.

Biological studies of leukemic blood have led to the reported demonstration of bacteria, and of other micro-organisms of undetermined nature. Although no specific micro-organism has been demonstrated, it is not to be hastily concluded that some infectious agents are not concerned in the etiology of the disease, or that the micro-organism isolated in particular cases was not the direct exciting cause in those cases.

It may very well be that the proliferation of leucocytes can be induced by various toxic agents, some of which are of bacterial origin.

Bacteria. Kelsch and Vaillard squeezed drops of blood from the fingers, and isolated therefrom a diplobacillus which was pathogenic for mice. The same bacilli appeared in the vessels of the tumors. Pawlowsky claims to have found one and the same bacillus in six cases, sometimes in the blood, or in the viscera, especially in the liver. In the blood they were found mostly in the plasma. In cultures on glycerin agar the bacillus produced a somewhat characteristic growth. Injected into rabbits this micro-organism could be identified morphologically in the blood for several weeks, but no symptoms of leukemia were produced. Although Pawlowsky believes he has discovered a specific microbe in the disease, he has not yet verified his results. Bonardi obtained cultures of *Staphylococcus pyogenes aureus* and *albus* from the blood of two cases of splenic leukemia.

Inoculations. Mosler and Westphal injected leukemic blood into dogs and rabbits with negative results. Bollinger with the blood, and Gilbert, Cadiot, and Roger with the leukemic tissues of diseased dogs, failed to produce any significant effects by injections into healthy animals.

The injection of leukemic blood and of the fresh juice of the spleen into various animals was without results in the hands of Mosler and Westphal, Bollinger, Nette, Eichenbush, and Müller.[4] Nette injected defibrinated blood under the skin into the peritoneum, and into the veins of the ear, epigastrium, and marrow, with negative results. The juice of leukemic nodes was injected into animals without effect by Troje, Litten, and Gilbert. Negative cultures of blood were obtained in acute cases by Ebstein, Roux, Westphal and Mosler, Guttmann, Mayet, Eichhorst,[1] Pfeiffer, Litten, and Sittmann.

Micro-organisms of undetermined nature have been described in the blood, or in the leucocytes, or in metastatic deposits, or in cultures, by Klebs,[2] McGillavry, Osterwald, Mayet,[2] Roux, Bramwell, Fermi, and Klein.

None of these writers has been able to follow up his observations.

The observations of Mannaberg and of Löwit[2] point to the possible existence of *protozoa* in leukemic blood.

In 8 per cent. of the lymphocytes of a case of lymphatic leukemia Mannaberg found hyaline bodies 1μ to 4μ in diameter, exhibiting ameboid activity such as is seen in the quartan malarial parasites. They failed to exhibit a nucleus by Romanowsky's stain, but after fixation in Hermann's fluid they stained well with safranin.

In the peripheral blood and viscera in myelemia Löwit has described an ameba which he classes with the sporozoa and terms *hemameba leukemiæ magna*. In lymphemia he finds another somewhat different ameba, *hemameba leukemiæ vivax*, which is scarce in the blood, but abundant in the viscera. In some mixed cases both amebæ were present, and they were also seen in pseudoleukemia and in v. Jaksch's anemia. Löwit has found the same amebæ in leukemic animals, and claims to have transferred the disease from one animal to another.

The method employed by Löwit in the demonstration of the parasite of leukemia is as follows:

The blood smeared in a thin layer is heated one to two hours at 110° to 120° C., and stained one-half hour in a concentrated watery solution of thionin. After thorough washing in tap-water and drying in air, the specimen is laid for ten to twenty seconds in solution of iodine (I, 1; KI, 2; aq. dest., 300), again washed, dried, and mounted in balsam. The parasite in its various stages then appears green or olive-green, or greenish black, the basophile granules and degenerated products of nucleus and cell body bluish red or brownish red, the protoplasm of leucocytes and their granules yellowish or yellowish brown. The blood smear must be thin. The solution of thionin should be several weeks or months old, long exposed to sunlight, and preferably containing a considerable growth of micro-organisms which seem to assist in "ripening" the stain. In the absence of an old solution good results may be obtained with a mixture of the thionin solution, 30; Loeffler's methylene blue, 15; but the green color of the parasites may be obscured by a diffuse dark stain from the methylene blue. The fading which ordinary preparations suffer within a few days may be avoided by mounting the specimen in balsam which has been mixed with a solution of iodine in xylol until the balsam is of light yellowish color.

Löwit regards the greenish stain produced by his method as the specific reaction of the parasite, and finds reason to believe that this color is assumed by a substance identical with or closely related to cellulose.

Turk believes that the hemameba of Löwit is an artifact resulting from the partial solution and deformation of mast-cell granules by the action of watery dyes, while others he regards as nucleoli of leucocytes. He claims to have found similar bodies in the blood of healthy men and rabbits and in the lymphocytes of chlorosis. His attempts to reproduce the disease in animals by injections of leukemic tissues were uniformly negative. Kraus, Widal, Hirschfeld and Tobias, Bloch, Litten, and Michaelis (cit. by Turk[3]) agree in general with the conclusions of Turk, while Vittadini, although finding the bodies only in leukemic blood, could not convince himself that they were parasitic.

Pathological Changes in the Viscera. Only the portions of this extensive subject will here be considered which bear upon the pathogenesis of the disease.

Marrow. In the early stages of the leukemic process the lymphoid marrow exhibits a slightly lighter color and greater consistence than is normal. This change results from a *cellular hyperplasia*, which obliterates blood sinuses, diminishes the blood-content, and causes atrophy of fat cells. With this cellular hyperplasia of the marrow of ribs, vertebræ, etc., there is an extension of lymphoid marrow throughout the shafts of long bones, where also the marrow appears firm and light colored. While in early stages the marrow may retain a fleshy tint, in advanced cases it is almost invariably very light colored and firm. In some advanced cases sclerotic and degenerative processes may follow the cellular hyperplasia, the writer having seen considerable areas of connective tissue and foci of mucoid degeneration in the marrow of old cases. In acute leukemia the marrow may be strikingly puriform in appearance, though invariably less diffluent than pus. In one case of the writer's this resemblance to pus was so marked as to require microscopic section before the true nature of the change could be positively identified.

In myelocythemia the affection of the marrow has in all undoubted cases been distinct, and if Litten's case can be accepted, as is doubtful, the leukemic process may be limited to the marrow. Leube and Fleischer (1881), Eichhorst, and recently Ambros, Hirschlaff, Kormoczi, and Denning have attempted to show that the lesion in the marrow may be absent in lymphemia, but their cases seem to have been examples of marked terminal lymphocytosis, probably in pernicious anemia. In lymphatic leukemia it was early shown that the marrow may be very slightly involved (Heuck,[1] Fleischer, Penzoldt), but in most cases the usual extensive hyperplasia is observed and in some reported cases of lymphemia the lymphoid hyperplasia was almost exclusively limited to the marrow (Kormoczi, Walz Pappenheim, Hirschlaff, and Reed).

Neumann and Pappenheim[1] claim that lymphatic leukemia is always of myelogenous origin, and the latter observer suggests that if the lymphoid hyperplasia affects first or only the spleen or lymph nodes pseudoleukemia results, while in lymphemia the lymph nodes and spleen are only secondarily involved. This view is apparently disproved by Rosenfeld's case of lymphemia in which no change was discovered in the marrow, while the spleen and lymph nodes were greatly enlarged.

The early and extreme involvement of the lymph nodes and spleen in many cases is also strong presumptive evidence against such a view.

The microscopic examination of the marrow in leukemia shows that the hyperplasia affects the variety of cells seen most abundantly in the blood. In myelocythemia these cells are largely the neutrophile myelocytes, which are found in very excessive numbers, of very large size, and in mitotic division or in various stages of degeneration. In the same cases, also, there is almost invariably an excessive number of large mononuclear hyaline cells. Eosinophile and giant cells may long persist, but do not appear to take a prominent part in the hyperplasia.

In pure lymphatic leukemia the lymphocytes alone are found in the hyperplastic areas (Müller[2]) and eosinophile and neutrophile cells are very scarce.

It is held by some (Ponfick, Mosler,[2] Müller[3]) that the leukemic process may at one period of its course affect principally the lymphoid structures, at another the marrow, so that the blood may at one time exhibit the changes of lymphatic, at another those of myelogenous leukemia. Fleischer and Penzoldt's case illustrates such a variation, and Michaelis has described another with the same purport.

Recent studies of leukemia have failed to support this view, which is strongly contradicted by the nature of the lesions which involve a hyperplasia of particular cells.

The red blood cells of the marrow are not greatly affected in the early stages of leukemia, as is indicated by the frequent absence of marked anemia, although in very cellular areas they are diminished in number from the first. Later in chronic myelogenous cases the same changes are seen as in pernicious anemia, but the excessive numbers of megaloblasts are seldom so prominent. In some acute cases, especially those of lymphatic type, the writer has seen almost entire absence of nucleated red cells in considerable sections.

The *distribution of the lesion* in the marrow of lymphatic leukemia is often very irregular. In an acute case the writer has found entirely normal structures in the ribs and femur, while the usual hyperplasia was present in the bodies of vertebræ. Moreover, the lesion, at least in its early stages, appears to be focal rather than diffuse. These peculiarities render it essential that very complete examinations should be made before it can be positively claimed that leukemia can exist without lesions in the marrow.

Liver. The enlargement of this organ is usually referable to the presence of numerous metastatic growths which follow the course of development described for all secondary leukemic deposits, and to infiltration of capillaries with proliferating leucocytes. In myelocythemia the liver is seldom free from such deposits, but in the lymphatic type the infiltration is usually limited to the portal canals and intralobular capillaries, although it may produce circumscribed tumors.

It is of great interest to note that the liver in myelocythemia gives evidence of having resumed its embryonal function of developing red cells. Neumann and Heuck first called attention to the presence of considerable numbers of nucleated red cells in the hepatic capillaries. In Heuck's case they were distinctly more abundant than in the general circulation. The resemblance of the leukemic to the embryonal liver is completed in the presence of mitotic leucocytes and giant cells, as described by Neumann,[4] Müller, Michaelis, and others.

Spleen. The pathological process in the spleen consists in hyperplasia of large and small mononuclear cells. These cells appear in excessive numbers throughout the pulp cords, while the limits of the Malpighian bodies become less defined. As a result of this cellular hyperplasia, the sinuses are choked with cells, and hemorrhages, infarcts, degeneration, and necroses occur, which lead to a variety of gross appearances in the enlarged organ.

Regarding the exact nature of the cellular processes, it has been shown that in myelocythemia, while the majority of the cells in the spleen are hyaline, yet neutrophile myelocytes occur in moderate numbers. These are generally regarded as derivatives of the marrow brought by the blood stream, although Müller has once seen mitosis in a splenic myelocyte. Numerous giant cells are sometimes found, which Müller regards as originating *in situ*. For the increased number of large and small lymphocytes, the chief mode of origin must be mitotic or amitotic division *in situ*, especially in the Malpighian bodies, as has been demonstrated by Müller. Along with this excess of colorless cells the organ commonly contains an excess of blood, and the usual process of destruction of red cells may everywhere be observed.

Nucleated red cells are visible in smears of splenic pulp in most cases of myelocythemia. The writer has been unable to convince himself that they are more numerous than in the general circulation, or, if slightly more numerous, that they are not mechanically sifted from the blood. Heuck, however, found them in the splenic smears when they were absent in the general circulation, and concluded that the spleen in leukemia may resume its embryonal function of developing red cells.

Following the stages of acute hyperplasia comes a later period marked by continuous increase in the size of the organ and great increase in consistency, owing to fibroid changes beginning in the trabecula and often extending diffusely throughout the pulp. In such stages the cellular-content is comparatively diminished without any apparent effect upon the character of the blood.

In lymphatic leukemia the same course of events is observed, but the new cells are limited to the large and small lymphocytes. In this form of the disease the lymph follicles are apt to be enlarged.

The general appearance of sections of the spleen in myelocythemia, the congestion, the indiscriminate filling of sinuses with red and white cells, the destruction of Malpighian bodies, the prominence of fibroid changes, and the usual absence of areas of distinct leukemic deposits, strongly indicate that *the enlargement of this organ results largely from mechanical sifting of red and white cells from the circulation and subsequent inflammatory changes.* In lymphatic leukemia, however, the proliferation of lymphocytes is usually very marked, and the Malpighian bodies must be considered one of the most important primary seats of the disease. In some cases it may, perhaps, be the exclusive seat of the primary lesion (cf. Hirschlaff, Rosenfeld).

Lymph Nodes and Other Lymphatic Structures. Swelling of the lymph nodes is one of the earliest of observed symptoms, having in some lymphatic cases preceded all other signs for some months. They may reach a considerable size, that of a hen's egg, but seldom become excessively large. Their capsules are almost invariably intact, unless ulceration occurs. The stage of cellular hyperplasia is succeeded, here as in the spleen, by one of fibrosis with diminution in the number of cells.

In myelocythemia the smears of lymph nodes yield almost the same appearances as those of the spleen, except for a relative excess of lymphocytes. Neutrophile myelocytes are scanty, but here again Müller has found mitosis in myelocytes. On section the outlines of the lymph follicles are lost, and the tissue consists of a diffuse mass of mononuclear cells, large and small, resembling lymphocytes, and often seen in mitotic division. In some cases distinct proliferation zones appear in small foci where the multiplication of leucocytes is very active.

Although the sinuses are usually choked and indistinguishable, yet, according to Birch-Hirschfeld, most of *the lymph nodes of leukemia differ from those of pseudoleukemia, in the fact that substances injected into the capsules pass through the leukemic node into the efferent vessel, but in pseudoleukemia the enlarged nodes are impervious.* The writer has been unable to find, in the literature or in sections and smears of nodes from his own cases, any constant microscopic differences distinguishing the lymph nodes of the lymphatic from those of the myelogenous type of the disease. In the former the small lymphocytes are rather more numerous, and, of course, the hyperplasia is usually much more marked.

In addition to the lymph nodes, spleen, and marrow, all preexisting lymphoid structures may become hyperplastic, especially in lymphatic leukemia, and new-growths of lymphoid tissue have been found of nearly universal distribution. From all of these localities new leucocytes are undoubtedly contributed to the circulation.

Character of Metastatic Deposits. Although Rindfleisch, Cornil and Ranvier, and Zeigler, held that leukemic deposits arise by mechanical lodgement of increasing numbers of leucocytes from the blood, Virchow early maintained that these deposits arise *in situ*. It has since been shown that while the early minute collections of cells are *white cell thrombi*, the further growth of these masses takes place principally by mitotic division of these cells (Bizzozero, Müller, and Neumann). Bizzozero has pointed out that the structure of these metastases closely resembles that of lymph nodes with reticulum and sinuses, and is not at all that of an indiscriminate deposit of blood cells. He found in such deposits quite as many mitotic nuclei as in similar nodules of metastatic carcinoma. Leukemic deposits must, therefore, be regarded as conforming in many important respects to the laws governing metastatic neoplasms.

The lymph nodes and spleen when affected exhibit a hyperplasia of the lymphocytes, which choke the sinuses, obliterate the follicles, and cause an overflow of these cells into the blood and probably also into the lymph stream. In lymphatic leukemia the new cells are of small size; in myelemia the cells are either of small size or larger, and with a distinct mass of protoplasm about the nuclei. The writer has made several attempts to demonstrate neutrophile granules in the cells of the lymph nodes in myelocythemia, but without success. In chronic cases the stage of hyperplasia is regularly followed by one of fibrosis, which in the spleen greatly increases the size and consistence of the organ.

Pathogenesis. The conclusiôns regarding the pathogenesis of leukemia which seem to be warranted from the foregoing review of the etiology and pathological anatomy of the disease are, to a large extent, those formulated by Virchow and Neumann, whose original ideas have been partially readjusted from time to time to suit newly acquired facts.

Leukemia must now be considered a primary disease of the blood-producing organs. The essential process consists in an excessive hyperplasia of the myelocytes or lymphocytes, with secondary increase of leucocytes in the blood, secondary lesions in the blood-forming and other organs, and leading eventually to disturbances in the production of red cells and to pernicious anemia. In the myelocytic type, the marrow and the neutrophile myelocytes are involved, and the development of red cells is soon disturbed, probably by mechanical conditions, possibly also by disturbance of the normal differentiation of the primitive parent cell into red cells. In lymphatic leukemia the lymphoid structures (lymph nodes, spleen, lymph cells of marrow) are almost exclusively affected, and red-cell formation is less early disturbed. It appears that lymphemia may principally affect one organ (the marrow) at one time, and another organ (the lymph nodes) at a later stage. In lymphatic leukemia the spleen may take active part in the initial process and furnish many new lymphocytes to the blood, but in myelocythemia its behavior is largely passive, and it seldom becomes the seat of true leukemic new-growths, but may as usual furnish lymphocytes to the blood.

The attempts to explain the various alterations in the leucocytes in leukemia and the numerous changes which the cell types in the disease undergo, by reference to the theory that a primitive parent cell exists in the marrow and dominates these changes has not, in the writer's opinion, been very successful.

It does not appear that the existence of two very distinct types of the disease has been rendered in any serious degree uncertain. Several observers have claimed that while in chronic leukemia the more differentiated types of white cells, lymphocytes and myelocytes, are produced in excess, in acute leukemia it is the parent lymphocyte itself which is multiplied.

It is claimed also that the transformation of chronic leukemia of either type into acute is marked by a disappearance of the more differentiated cells and the appearance of the earlier primitive lymphocyte (Frankel, Wey), and that in acute leukemia we have a point of union of the leukemic types.

This conception, while favored by many important theoretical considerations, is still largely conjecture unsupported as yet by definite observations. There are as yet no morphological criteria on which to identify the primitive lymphocyte, nor is the normal location of this cell clearly determined. It may be true that cases of acute leukemia exist in which the primitive parent cell appears in excess, but the morphology of the large cells in acute leukemia indicates that in the great majority of cases these large cells still fall into two rather distinct classes, viz., large hyaline or basophile mononuclear cells without a trace of granules, and large myelocytes, more or less deficient in granules. In myelocythemia the cells are considerably removed from the parent cell, and the deficiency of granules is most reasonably referred to the degenerative changes associated with rapid multiplication.

In lymphemia the morphology of the cells oscillates between that of distinct lymphocytes and that of the larger, pale, hyaline cells, derived from marrow, spleen, and lymph nodes, for which no one can claim a capacity to develop into any other cell than the differentiated lymphocytes.

It thus appears most probable that the hyperplasia of leukemia affects certain derivatives of the parent lymphocytes which may closely approach each other in morphology, but that the separation of two types of leukemia always remains distinct. The relation of a primitive parent cell to leukemia remains for future observations to determine, and this can probably not be successfully accomplished by theoretical deductions, as some have attempted to do, but only by actual study of the concrete facts presented by the disease.

The importance of *proliferation of leucocytes in the circulation* has been maintained, especially by Biesiadecki and Löwit,[1] who believe that leukemia is a *primary disease of the blood.* While nearly all experienced observers agree that leucocytes multiply to some extent in the circulation, there is almost equal agreement that the extent of mitotic division in the blood is inconsiderable compared with that in the viscera.

The leukemic deposits of the viscera develop by proliferation of original thrombi of leucocytes, which under favorable conditions undergo active mitotic division, develop a supporting stroma, and behave in essential particulars like true neoplasms, while probably continuing to furnish new leucocytes to the blood.

The red cells during the early stages of myelemia suffer from the disturbances of development, such as exist in chlorosis. In later stages red-cell production reverts to the embryonal megaloblastic type, mitotic megaloblasts are seen in the marrow, spleen, and liver, and the changes of pernicious anemia are established in the blood.

The partial resemblance of leukemia to a neoplasm affecting the lymph nodes, spleen, marrow, or blood was early recognized, and this theory of origin has been discussed by most writers and openly maintained by Kottman, Bard, Herzfeld, and lately by Gilbert, who compares lymphemia to a sarcoma affecting lymphocytes.

To what extent the origin and course of leukemia follows the laws known to govern neoplasms is an interesting inquiry, which cannot here be pursued at length, but which may well be kept in mind. According to such a theory it is necessary to suppose that lymphatic leukemia is a tumor originating in the lymphocytes, myelemia a tumor affecting myelocytes.

There are, however, other tumors affecting lymphocytes, the lymphosarcomata, which behave in a very different manner from lymphemia in growth, and especially in their metastatic characters. The cases of lymphemia arising in the course of lymphosarcoma are here of great interest (Palma). Leukemia has also been found associated with carcinoma (Lannois and Regaud), and with endothelioma of lymph nodes in a lymphatic case studied by the writer.

Changes in the Blood.

The *bulk* of blood is apparently little altered by the uncomplicated leukemic process, but in autopsies on chronic cases the same diminution in the total quantity of blood may be noted as in pernicious anemia, and from the same cause.

The *color* of the blood may in well-marked cases be entirely normal, as anemia is of slow progress. Usually it is slightly lighter in color and more fluid, owing to pronounced anemia and great excess of leucocytes. The exuded drop has been described as lymphoid or puriform, but when properly expressed blood of such character must

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be extremely rare. When the red cells are below 1,000,000, and the leucocytes almost equally numerous, the blood drop still resembles blood, though of very light color. When obtained in bulk at autopsy the blood coagulates slowly, the red cells settle to the bottom, followed by a characteristic thick layer of leucocytes and fibrin, while the very clear serum floats above. In the cadaver deposits of leucocytes resembling abscesses may be found in various situations, often in the heart and pulmonary vessels.

The *coagulability* is greatly diminished and may be entirely lost, but less often than in fatal pernicious anemia.

To the *touch* the blood may have a cohesive mucous quality, as noted by Grawitz.

When leukemic blood is smeared and dried on a glass slide, it exhibits a peculiar granular opaque appearance which is readily identified as belonging to a great excess of leucocytes.

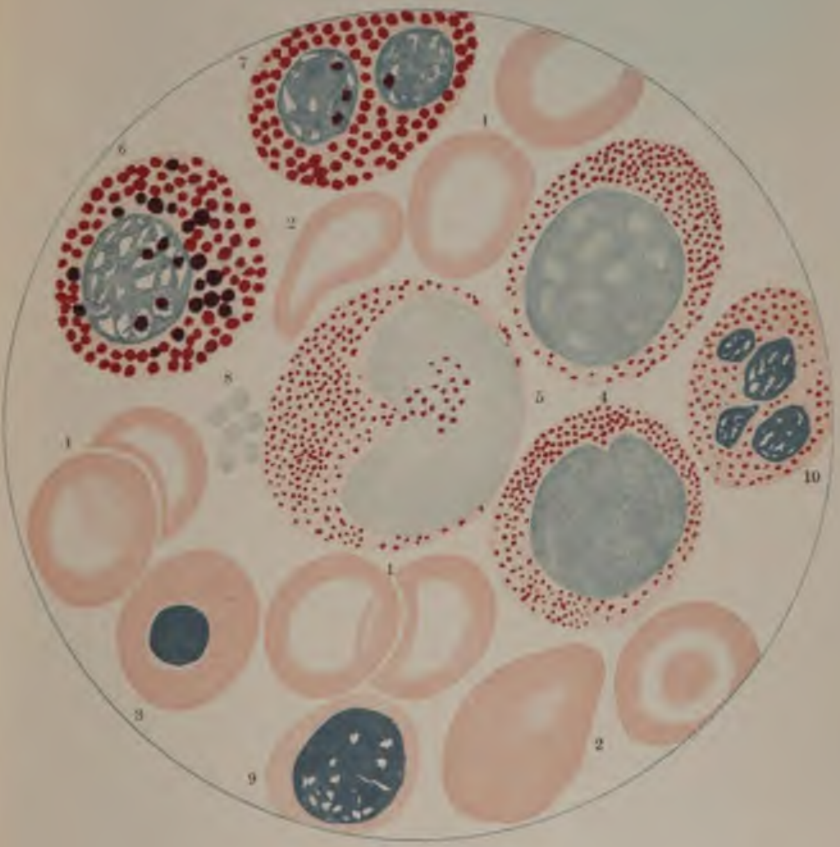
The Red Cells. In average chronic cases the red cells are usually reduced to 2,000,000 to 3,000,000, falling to 1,000,000 or lower in fatal cases, but not usually reaching the extreme reductions seen in pernicious anemia. On the other hand, Cabot reports a case with over 5,000,000 red cells, 134,000 white, Hb 78 per cent. At death the numbers of red cells depend upon the presence or absence of hemorrhage, and upon the length and character of the disease. There are no marked differences in the grade of anemia in lymphocytic and myelocytic leukemia.

Morphological changes in the red cells are invariably present. In the average chronic case the uniform loss of Hb in red cells showing normal rouleaux and very uniform size and shape, with a rather free admixture of normoblasts, gives to the stained blood smear a very peculiar and almost pathognomonic character. Possibly the abundance of leucocytes controls the movements of the red cells in the smearing process, but from some cause the appearance of the red cells in stained specimens of leukemic blood is very characteristic. (See Plate VIII.) At this early stage the normoblasts are frequently present in greater numbers than in any other similar condition.

Later the red cells begin to show the changes of more severe chlorotic anemia, differences in size and shape appearing, degenerative changes occurring with greater frequency and extent, until in many old severe cases the features of pernicious anemia are fully established. Even in such stages, however, the appearance of the red cells usually differs distinctly from that of pernicious anemia in the more uniform loss of Hb and in the larger number of nucleated red cells, among which a few are almost invariably normoblasts. Mitotic nucleated red cells are rare. In most other respects the same changes are seen as in pernicious anemia.

Hemoglobin. Estimates of Hb in leukemia are at present somewhat unsatisfactory, owing to the opacity produced in the diluted blood by an excess of leucocytes. In the early stages of the disease the loss of Hb follows the reduction in red cells, as in chlorosis, a diminution being demonstrable at times before any change in the number of red cells, and the Hb-index remaining about 0.5 to 0.6

PLATE VIII.



Myelogenous Leukemia. Triacid Stain.)

Figs. 1. Normal-sized red cells deficient in Hb.
Figs. 2. Pear-shaped poikilocyte.
Fig. 3. Normoblast.
Fig. 4. Myelocytes (Ehrlich's).
Fig. 5. Myelocyte (Cornil's).
Fig. 6. Myelocyte (eosinophile).
Fig. 7. Eosinophile leucocyte (normal).
Fig. 8. Blood plates.
Fig. 9. Lymphocyte.
Fig. 10. Polynuclear leucocyte, nucleus subdivided.

Later the estimates are untrustworthy, but the Hb-index probably rises.

The Leucocytes. The white cells are usually so much increased as to leave no doubt, *from their numbers alone*, as to the nature of the disease. In cases of very moderate severity there are usually from 100,000 to 200,000 leucocytes; Cabot's average of first examinations in 39 cases was 438,000. Rarely 1,000,000 or more white cells are present in the cubic millimetre of expressed blood, but it appears doubtful if any such excessive number actually exists throughout the circulation.

As a rule, the severity of the general condition is proportionate to the increase of leucocytes, except in acute leukemia, when the number of leucocytes may not exceed that of inflammatory leucocytosis.

Variations in the number of leucocytes may be observed at different periods of the day, as shown by Hayem, who found 122,500 at 10 A.M., and 235,000 at 4 P.M. of the same day. These rapid changes must be referred largely to vasomotor disturbances leading to unequal distribution of cells, or, possibly, to a sudden but temporary increase of the leucocytes discharged from the marrow. Other variations in number of leucocytes will be considered under the course of the disease.

Morphology of Leucocytes in Myelemia. 1. NEUTROPHILE MYELOCYTES. These are *large mononuclear cells with neutrophile granules.* It is of some moment to distinguish two varieties of neutrophile myelocytes in leukemia.

(*a*) Cells of about the same size as normal polynuclear leucocytes, with well-staining, central nucleus, as described by Ehrlich and Utheman. These cells are abundantly present in leukemic blood and are generally the only form of myelocyte seen in secondary anemia. (Plate VIII., Fig. 4.)

(*b*) Cornil and H. F. Müller have called special attention to the *large myelocyte with pale eccentric nucleus.* This cell is not usually seen except in leukemia, but in some other conditions, especially in the secondary anemia of children, it may appear in moderate numbers. These cells have frequently been seen in mitotic division. (Plate VIII., Fig. 5.)

As first shown by Mosler, and abundantly verified by later observers, the presence of large numbers of neutrophile myelocytes is pathognomonic of leukemia. In 28 cases (chronic?) Cabot found that between 20 to 60 per cent. (average 35 per cent.) of all leucocytes present were myelocytes. With the extreme leucocytosis of chronic leukemia there are nearly always enough myelocytes present to establish the diagnosis as against any other condition that has yet been observed.

In acute myelocytic leukemia, however, this rule may fail and both the numbers and proportions of myelocytes may not exceed those seen by Engel in fatal diphtheria.

Of recent cases of acute leukemia in which the type of the disease was satisfactorily determined, Frankel (1895) could find only three of myelocytic type, the great majority being of the lymphatic variety. In 1897 the writer saw three cases of rapidly fatal leukemia, verified by autopsies, and all of the myelocytic type. They occurred in the services of Drs. Thomson and Delafield, at Roosevelt Hospital. In two of these the changes in the blood were from the first observation typical of the condition and the marrow was puriform, while the spleen and lymph nodes were but slightly affected. In the third case, on the first examination, with a leucocytosis of ordinary inflammatory grade, 5 per cent. of the cells were with difficulty recognized as myelocytes. Eosinophile myelocytes and normoblasts were absent, and there was then not sufficient ground on which the diagnosis of leukemia could rest. Later, the leucocytosis and the proportion of myelocytes increased and the diagnosis of acute leukemia, made with reserve just before death, was fully verified at autopsy.

In this case, on account of the small numbers and indistinctness of the granules in the myelocytes, as well as the moderate grade of leucocytosis, the writer did not feel certain of the diagnosis until after the microscopic examination of the bone-marrow.

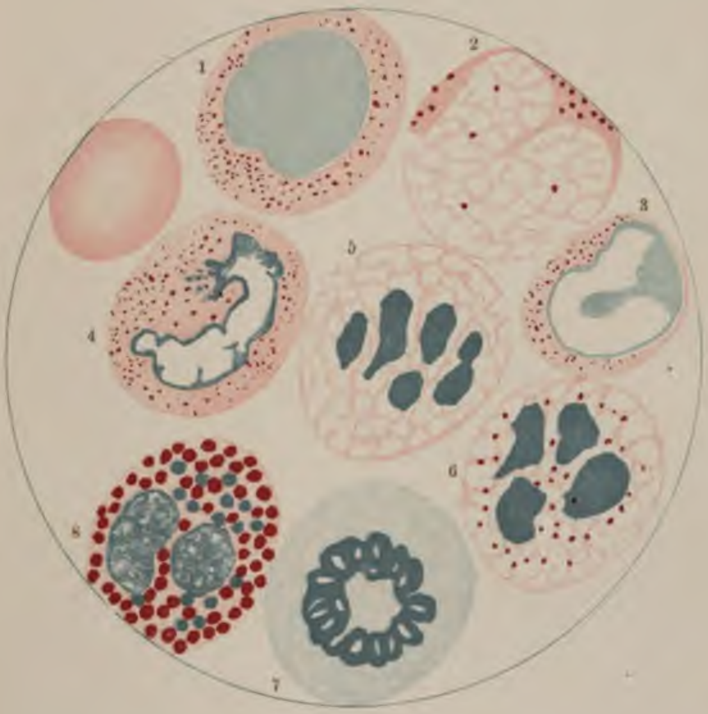
Degenerative Changes in Myelocytes. The myelocytes of leukemia are usually deficient, *and may be entirely lacking,* in neutrophile granules. In the latter case they are indistinguishable from large lymphocytes, except by the great pallor of their nuclei (triacid stain). Such forms occur especially in acute leukemia. The nuclei of degenerating myelocytes may undergo hydropic degeneration. It is then practically impossible to determine the origin of these altered cells. (Plate X., Figs. 1 and 2.) Some very small mononuclear cells with neutrophile granules seen in leukemic blood Ehrlich regards as the result of subdivision of polynuclear leucocytes (neutrophile pseudolymphocytes).

2. POLYNUCLEAR NEUTROPHILE LEUCOCYTES are excessively numerous in myelocythemia, but are commonly found in diminishing proportions, though in increasing numbers, as the percentage of myelocytes increases. Cabot, using the triacid stain, found between 17 and 72 per cent. (average 46 per cent.) of polynuclear leucocytes. Even when the myelocytes are relatively few their presence seems always to be at the expense of polynuclear cells, a fact which indicates a slower progress to full development as well as increased production of these cells in the marrow. The most successful demonstration of nuclear figures in these cells (*e. g.*, by Nocht's stain) show very numerous transitional forms between the spheroidal and the multilobate nucleus, while after the triacid stain it is usually impossible to distinguish between many single and polymorphous nuclei in neutrophile cells.

In lymphatic leukemia polynuclear leucocytes are usually scarce and may not be found at all.

Degenerative changes in the polynuclear leucocytes are very common and very marked. Their cohesiveness is increased and they appear in large inseparable groups with myelocytes. Their nuclei are usually pale (karyolysis) and may become excessively faint. Gumprecht suggests that this pallor of the nuclei of neutrophile cells indicates the transformation of the nucleins into xanthin bodies; or the lobes may become more numerous, entirely separate, very compact, and densely staining (pyknomorphous), while the granular cytoplasm is replaced by homogeneous, highly refractive material (Plate X., Figs.

PLATE X.



Degenerating Leucocytes in Myelogenous Leukemia.
(Triacid Stain.)

Fig. 1. Myelocyte deficient in neutrophile granules.

Fig. 2. Necrotic myelocyte, complete karyolysis; hydropic degeneration; loss of neutrophile granules.

Fig. 3. Degenerating myelocyte. Hydrops of nucleus.

Fig. 4. Polynuclear leucocyte deficient in neutrophile granules; hydrops of nucleus.

Figs. 5, 6. Polynuclear leucocytes. Loss of neutrophile granules. Advanced subdivision of nucleus.

Fig. 7. Myelocyte in mitotic division. (Eosin and methylene blue.)

Fig. 8. Eosinophile leucocyte. Some granules basophilic. (Eosin and methylene blue.)

5 and 6); or the nuclei may undergo hydropic degeneration. (Plate X., Fig. 4.) All grades of deficiency of granules may be observed, but the writer has been unable to demonstrate fat in the neutrophile cells of leukemia. Considerable variations in the size of these cells may be noted in some cases of leukemia.

3. EOSINOPHILE CELLS are usually much increased in number in myelocythemia, but their proportions to other forms of leucocytes commonly vary within normal limits. In some cases, however, the proportions are increased, but never so much as in cases of pemphigus, etc. Their proportions are not pathognomonic of the disease, *but in most cases their total numbers greatly exceed those found in any other condition.* Ehrlich places their numbers between 3,000 to 100,000 per c.mm., thereby practically demonstrating, as he originally claimed, that the excess of eosinophiles is among the pathognomonic signs of the disease. In some specimens of blood the eosinophile cells vary greatly in size, some of them being very small, while their neighbors are hypertrophic. A few large basophilic granules may be found in the polynuclear as in the mononuclear eosinophiles of leukemia. These cells are actively ameboid.

In acute leukemia of both types and in chronic lymphocythemia eosinophiles are scarce or absent.

Eosinophile myelocytes are mononuclear cells with eosinophile granules.

In some of these cells the granules are of uniform size and staining quality, or there may be some basophile granules among the eosinophile, or the granules may vary greatly in size and in density of stain. *Eosinophile myelocytes with granules of very unequal size and density of stain are, in the writer's experience, pathognomonic of myelocythemia.* (Plate VIII., Fig. 6.) Bignami, however, speaks of the occurrence in pernicious malaria of eosinophile myelocytes "such as are seen in leukemia."

In the majority of cases of myelocythemia eosinophile myelocytes constitute a large proportion of all eosinophile cells present. In lymphatic leukemia they are almost always absent, and in acute leukemia they are scarce or absent.

4. LYMPHOCYTES. The numbers and proportions of lymphocytes in myelocythemia vary in different cases and at different periods in the same case, and one must be prepared to find marked variations in the proportions of lymphocytes without being able to attach much significance to such changes. As a rule, the lymphocytes are much increased in numbers, while diminished in relative proportions to the neutrophile cells. The increase of lymphocytes appears to have no uniform relationship to the stage or character of the disease, as small numbers are found at early stages of some cases, and large numbers in the late stages of others, and *vice versa*. Cabot found an average of 10.6 per cent., but when at their lowest (2 per cent.) they were still more numerous than in normal blood. The proportions of large and small lymphocytes also vary without apparent relation to the other features of the disease. Usually but not always the large cells outnumber the small. It is probable that many myelocytes

deficient in neutrophile granules are commonly enumerated among large lymphocytes, especially in acute myelocythemia. Turk's "Reizungsformen," mononuclear cells with opaque, densely staining cytoplasm, are also commonly included among the large lymphocytes. They are probably ancestral forms of megaloblasts. The access to the blood stream in myelocythemia of large numbers of true lymphocytes, small and large, may be referred to a secondary, but, nevertheless, continuous hyperplasia of these cells in the marrow and lymphoid tissues, and to accidental and temporary factors which excite their proliferation and attract them to the blood. Their presence, even in considerable numbers, cannot at present be regarded as an indication that the disease is of mixed type.

5. LARGE MONONUCLEAR LEUCOCYTES, with very faint cytoreticulum and vesicular nucleus, are seen in considerable numbers in most cases of leukemia, but they appear to lack special significance. They are very apt to suffer damage in the smearing process and to appear in the dry specimen as large, coarsely reticular nuclei, without demonstrable cell body. (Plate IX., Fig. 3.) These cells may contain granules giving the reaction of glycogen. The larger forms may show horseshoe-shaped nuclei. In the cases of acute leukemia described by Frankel and others, the majority of cells were large mononuclear leucocytes.

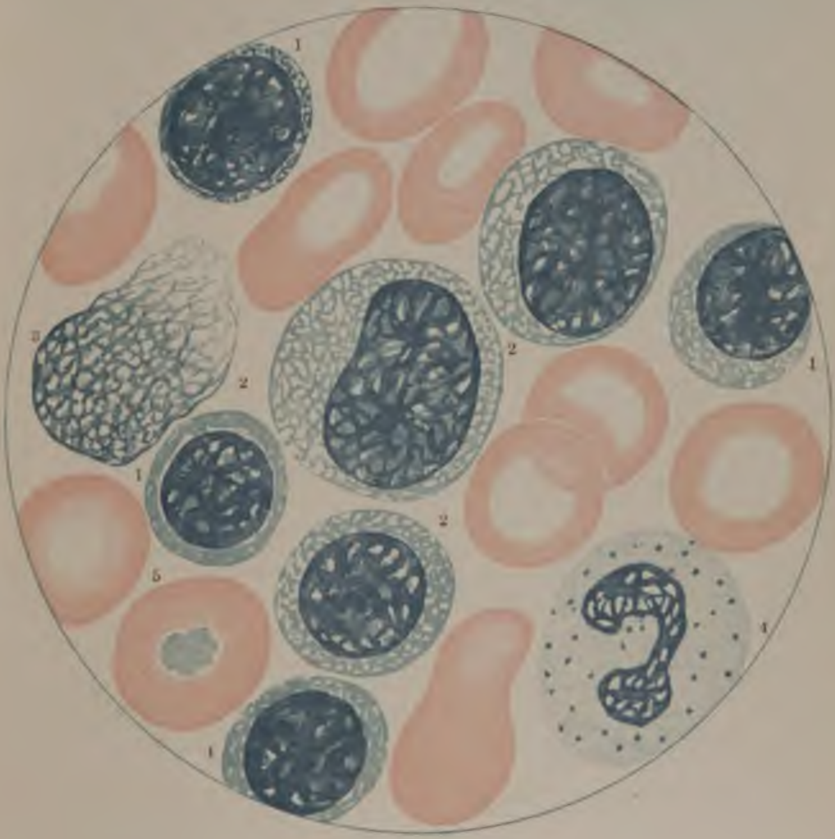
Degenerative changes are noted in the small and often in the large lymphocytes of leukemia. The nuclei of the small cells, instead of remaining compact, may become incurved and finally bilobed or trilobed, while the cell body remains basophilic (Rieder, Ehrlich). Litten[2] describes, in two acute cases, very large cells in which the nucleus was obscured by many globules of fat blackened by osmic acid. There are some other reports of fatty degeneration of leucocytes in leukemia, but apparently none from very recent literature. Mitotic figures in lymphocytes of lymphemia are reported by Wertheim.

6. MAST-CELLS. These cells are very constantly increased in chronic myelocythemia, but in some cases a prolonged search is required for their discovery. Although occasionally seen in other conditions, in leukemia they are usually so markedly increased as to constitute a very reliable diagnostic feature of the blood. They are at times more abundant than the eosinophile cells. They are usually absent in lymphemia, and the writer has failed to find them in acute myelocythemia.

Strauss found 5 per cent. of mast-cells among the leucocytes in blister fluid from a case of myelocytic leukemia, and Milchner reports 23.9 per cent. of mast-cells in the sediment from ascitic fluid in a similar case. In the blood of one case Taylor reports the remarkable number of 140,000 mast-cells.

Special Characters of the Blood of Lymphatic Leukemia. In this type of the disease the lymphocytes are the only form of white cell appearing in the blood in increased numbers. In cases in which a positive clinical diagnosis is possible, the nature of the condition is at once evident from the great abundance of these cells, but owing to

PLATE IX.



Lymphatic Leukemia. (Eosin and Methylene Blue.)

Figs. 1. Small lymphocytes.
Figs. 2. Medium-sized and large lymphocytes.
Fig. 3. Degenerating basket-shaped nucleus of large lymphocyte, without cytoplasm.
Fig. 4. Polynuclear leucocyte. Nodal points of cytoreticulum.
Fig. 5. Red cell. Polychromasia of Maragliano.

the numerous other causes of chronic lymphocytosis it is at present impossible to state what is the lowest proportion of such cells seen in the blood of genuine lymphemia. In well-marked cases they are quite as numerous as the leucocytes of myelocythemia, while some of the highest counts on record have been reported in this type of the disease.

Usually the lymphocytes are of small size and normal structure, and the writer has specimens of one very marked case, in which almost all the leucocytes were small mononuclears. Their percentage commonly runs between 80 and 90.

In rather rare cases reported by Frankel, Grawitz, Cabot, and others, and in one child observed by the writer, the great majority of the cells were unusually large and their cytoreticulum faintly staining. Between the two extremes are cases showing various proportions of small, medium-sized, and large lymphocytes. The small lymphocytes are usually more abundant in chronic cases and in adults, the larger cells tending to become prominent in acute cases and in children. Gerhardt refers to a case in which large lymphocytes, abundant in the early acute stage, were replaced during a chronic course of six months, by small lymphocytes.

Frankel believed that these cells could be regarded as pathognomonic of acute lymphatic leukemia, but his claims have been fully disproved. Large mononuclear cells with hyaline bodies were common in the writer's cases of acute myelocythemia, and reasons have been given to show that some of these cells may result from loss of neutrophile granules of myelocytes. Askanazy insists that the large cells of Frankel are not lymphocytes, but myelocytes deficient in granules, a view to which the writer is inclined from the study of three cases of acute leukemia. Hirschlaff has recently reported two acute cases in which most of the leucocytes were large mononuclear cells without granules. In a moderate proportion of them neutrophile granules and in a larger number eosinophile granules were noted.

This variation in size of lymphocytes may have an anatomical basis in the structure of the hyperplastic lymph nodes, as in some cases these nodes contain mostly small lymphocytes, at other times large mononuclear cells. (Birch-Hirschfeld, Benda)

These and other considerations oppose the interesting view recently expressed by Rosenfeld, that when the lesion of lymphemia is in the marrow many large lymphocytes appear in the blood, anemia rapidly develops, and the disease runs a severe course, while if the lesion affects only spleen and lymph nodes large lymphocytes are absent from the blood and anemia is of slow development.

Myelocytes, both neutrophile and eosinophile, and mast-cells are usually absent or extremely scarce in lymphemia, but are occasionally seen in scant numbers (Müller). Polynuclear leucocytes, both neutrophile and eosinophile, are also comparatively scarce.

The red cells suffer the same changes as are seen in myelocythemia, but nucleated red cells are usually very scanty and may not be found at all. Da Costa, however, found 10,678 nucleated red cells in one case, the great majority normoblasts.

Variations in the Blood Changes in Leukemia. Both the numbers and the proportions of leucocytes in leukemia are subject to considerable variations from many causes.

1. **Intercurrent Diseases.** A considerable number of cases have been reported showing that intercurrent infections may greatly alter the appearance of the blood in leukemia. These cases have been collected by Frankel and Marischler, Froelich, Cabot, Kormoczi, and McCrae. Some of the reports refer to *terminal septicemia* which has resulted usually in a marked and rapid decrease of leucocytes, usually with an increase in the proportion of polynuclear cells. Frankel, who saw the leucocytes fall from 220,000 to 1200, refers this result to pure leucocytolysis. Such cases have been observed after typhoid fever, pneumonia, empyema, erysipelas, septicemia, tuberculosis, carcinoma, and influenza (Kovacz). Beitzke, from observations in sixteen cases, concludes that all diseases which cause polynuclear leucocytosis tend to increase the proportion of these cells in leukemia. Distinct changes in leukemic blood do not invariably result from such intercurrent diseases. In cases where the infection is localized (empyema, erysipelas) the normal activities of the marrow seem to be stimulated, and while the leucocytes diminish the proportion of polynuclear cells increases (Freudenstein, Kovacz, Müller[4]). Kraus has recently reported a reduction of from 393,000 to 4000 leucocytes in a few days as the result of double pneumonia and empyema. The viscera (marrow, liver, spleen) showed no evidence of leukemic infiltration, and Kraus suggests that the suppurative process had not only transformed the blood, but had resolved the essential visceral lesions as well. Usually intercurrent infections diminish the volume of spleen and lymph nodes even when, as in Müller's case, the leucocytes are increased. After the subsidence of the infection the blood soon resumes its original condition (Grawitz), or even before the infection subsides the first effect may pass off, as occurred in Eisenlohr's case, within fourteen days.

Although no permanent improvement resulted in any cases suffering from intercurrent infections, several attempts have been made to favorably influence the course of leukemia by artificial leucocytosis. Jacob reduced the leucocytes from 850,000 to 282,000 in a few days by repeated injections of glycerin extract of spleen, but could not report any improvement in the patient. The same result was obtained by Richter using *spermin*. Richter and Spiro claim to have increased the leucocytes in leukemia from 170,000 to 560,000 within three hours after injection of cinnamic acid, followed by prompt return to the previous condition. Heuck[2] has also reduced the leucocytosis of leukemia by injection of tuberculin. In mild inflammatory processes there may be no effect upon the leucocytes of leukemia, as indicated by the cases of Richter and Heuck.[1]

Antemortem leucocytosis of considerable degree (172,000) was observed by Thorsch in a case complicated by pneumonia. In a case of lymphatic leukemia dying of septicemia, Müller found, four days before death, 400,000 leucocytes when there had previously been but 180,000. A differential count was not made, but Müller regarded the increase as referable to a *polynuclear leucocytosis*.

Chronic infections have much less effect in altering the character of leukemic blood. Quincke and Stintzing reported a general improvement of the leukemic process during acute miliary tuberculosis and during an exacerbation of chronic phthisis.

In a case of lymphatic leukemia dying with carcinoma of the kidney Marischler found a decrease of leucocytes from 96,000 to 48,000, with marked increase in the proportion of polynuclear cells.

2. **Spontaneous Changes in the Blood of Leukemia.** A few instances are recorded in which the disease, as indicated by the blood changes, appeared to become transformed from one type into another. The first of these cases was that of Fleischer and Penzoldt, who observed splenic leukemia pass into lymphatic. It seems possible that this case may be placed with others described by Frankel[2] and Gerhardt, in which lymphatic leukemia began acutely with large lymphocytes, but progressed more slowly with small lymphocytes. Wey observed a case of myelocythemia in which, within ten weeks, the polynuclear cells fell from 33.5 per cent. to 3.7 per cent., the mononuclears rose from 66.5 to 96.3 per cent., among which were neutrophile myelocytes, large hyaline cells, and a few lymphocytes. This and Reiman's case represent a series of transformations sometimes observed, in which the disease begins with a considerable proportion of polynuclear neutrophile cells, passes through a stage in which myelocytes exist in high proportions, and ends in more or less fulminant form, with many large cells deficient or entirely lacking in neutrophile granules.

Gerhardt observed the blood of a case of leukemia of marked grade pass into that of pernicious anemia, the excess of leucocytes disappearing in three days. The possible transformation of pernicious anemia into leukemia has already been discussed.

Pseudoleukemia has been described as changing into leukemia in several cases. (See Pseudoleukemia.)

In view of the occasional reports of cases of leukemia in which the diagnosis rested upon the study of cadaveric blood, reference may be given, but with some caution, to the report by Seelig, of a case in which during life many myelocytes but few lymphocytes were found, while in the blood of the cadaver only lymphocytes were encountered.

Chemistry. **Specific Gravity.** The specific gravity of the blood is usually reduced, owing principally to the loss of Hb. But leukemia is one condition in which Hb is often replaced by other albumins, so that the gravity of the blood is relatively high in comparison to the Hb-content, and in exact proportion to the increase of leucocytes. Thus Diebella reports a case with 2,600,000 red cells, 750,000 leucocytes, and specific gravity of 1.060. The lowest observations are placed by Grawitz at 1.036. The low figures are usually seen in cases complicated by hemorrhages or other causes of secondary anemia. The specific gravity of the serum has been found by Grawitz and by Taylor to fall within normal limits, 1.023 to 1.030.

Alkalescence. The alkalescence of leukemic blood has been found by v. Jaksch, Peiper, and others to be much diminished. Early observers found the blood distinctly acid soon after death (Scherer), probably owing to the postmortem formation of acids. v. Noorden referred the diminished alkalinity to the development of acids during life. Lactic and formic acids have been isolated from the fresh blood by Scherer, Mosler, and others, and acetic acid, after death only, by Mosler.

Albumins. Fibrin has been found in excessive quantity, 5.7 per cent. (normal 0.25 per cent.), by Parkes, but in chronic cases with severe anemia it is diminished (Robin).

Peptone. Considerable interest attaches to the demonstration, first by Bockendahl and Landwehr, of considerable traces of peptone in the splenic pulp and in the blood of leukemia. This observation has lately been verified by Mathes, who, however, has shown that the principle in question is not Kuhne's peptone, but deutero-albumose. Freund believes that the retarded coagulation of leukemic blood is largely referable to the presence of albumose. The products of the excessive destruction of leucocytes have been traced in the presence of their various derivations in blood and urine.

Nucleo-albumin and **deutero-albumose**, but not peptone, have been isolated from the serum by Mathes; mucin or a closely allied substance by Scherer and others; and a principle resembling gluten by Salkowski.

Xanthin bodies, which represent further decomposition products of leucocytes, have been found in the spleen and blood by Scherer, and many others The xanthin bodies of Kossel are more abundant and more easily recognized in leukemic than in normal blood.

Uric acid has been found in traces by Mosler, Fowlwarczny, Klemperer, and Weintraud. An excessive excretion of uric acid in the urine of leukemia has often been observed, and Magnus-Levy reported as high as eight grains daily, but since the most abundant appearance of leucocytes need not indicate the time of their greatest destruction, there is no parallel between the excretion of this principle and the excess of white cells in leukemia (Minkowski, Wey). Gumprecht[2] finds that in those cases of leukemia in which uric acid excretion is normal there is an increase of "alloxurkorper," which consists of alloxan, urea, uric acid, and other principles of similar significance with uric acid.

Glycogen. An increased quantity of *glycogen* has been extracted by Salomon and by Gabritschewsky. The fat-extract has been found distinctly increased by Robertson, Isambert (0.72 per cent.), and Freund and Obermayer.

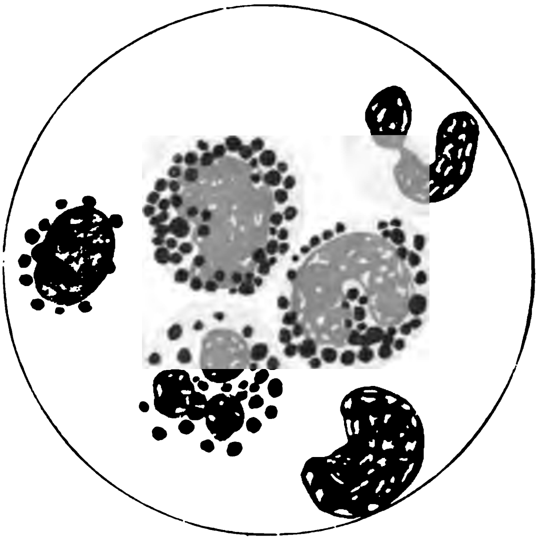
Tyrosin was isolated by Fowlwarczny, and **lecithin** and **cholesterin** from the blood of a hematoma by Freund and Obermayer.

Of **inorganic principles** the iron has uniformly been found diminished (2.24 to 2.97 per cent. of ash), usually in proportion to the loss of Hb (Strecker, Scherer, Freund, and Obermayer).

Phosphorus, *sulphur*, and *sodium* were much increased and *potassium* and *chlorine* much diminished in Freund and Obermayer's[2] case, while the total salts were moderately increased.

Charcot-Leyden Crystals. Robin first observed these peculiar crystals in the tissues of a leukemic cadaver, and they were described by Charcot and Robin in 1853. Zenker later (1855) claimed priority in their discovery. They were found in all parts of the circulation in a case examined after death by Wallace in 1855. Later they

PLATE XI.



Mast Cells. (Ehrlich's Dahlia-stain.)

were carefully studied by Charcot and Vulpian, who described them as colorless, refractive, elongated octahedra, 0.016 × 0.005 mm., or occasionally of much larger dimensions, insoluble in cold water, alcohol, ether, or glycerin, soluble in hot water and in most acids and alkalies. After death they appear in the blood, exudates, and especially in the spleen, and their numbers increase with post-mortem changes in the tissues. Like most other crystals of post-mortem formation, they are often seen in or on the leucocytes. Their occurrence in sputum and relation to eosinophile cells was pointed out by Charcot and Leyden. According to Neumann,[1] they are found in leukemia only when the blood contains many large cells with abundant cytoplasm and large nuclei, and hence are not seen in lymphatic leukemia. Chemically they were regarded by Charcot as albuminates, by Salkowski as mucinous. Lately Pohl has shown that they are identical with Bottcher's spermin crystals, which are a product of the destruction of the nucleins of disintegrating cells, and probably consist of spermin phosphate. Scherer also concluded that they are composed of phosphoric acid and an organic base.

They were obtained in the blood drawn during life by Neumann and later by many others.

They are not peculiar to leukemia, but appear in the sputum of asthma, in feces and mucus surrounding intestinal parasites, and have been found in the fetal blood in simple anemia (Gowers).

Lewy states that eosinophile granules may be thrown down in the form of Charcot-Leyden crystals by fixation of tissue containing eosinophile leucocytes in magnesium sulphate, 1 part; water, 7 parts; or sodium sulphate, 1 part; water, 10 parts. The tissues thus fixed should be thoroughly washed in water and hardened in alcohol.

The Diagnosis of Leukemia. In no other department is the examination of the blood to be interpreted with greater certainty in one case or greater reserve in another than in its application to the various types of leukemia.

1. *The changes in the blood may yield positive diagnostic signs of leukemia.*

This result is obtained in the great majority of cases of both types, whether acute or chronic.

These signs are briefly:

In Myelocythemia. An excessive leucocytosis (150,000 to 1,000,000).

A large number and considerable proportion of neutrophile myelocytes (20 to 60 per cent.).

A large number of eosinophile cells (3000 to 100,000) of which many are mononuclear and exhibit very large densely staining granules.

An excessive number of polynuclear neutrophile cells. Characteristic changes in the red cells.

To these may be added: The presence of many mast-cells, many nucleated red cells, mitotic nuclei in leucocytes, extreme and peculiar degenerative changes in leucocytes.

In Chronic Lymphocythemia. An excessive leucocytosis (150,000 to 1,000,000).

The presence of 85 to 99 per cent. of lymphocytes.

Scanty numbers of myelocytes, eosinophiles, nucleated red cells, and mast-cells.

For the positive diagnosis of either type of the disease the essential point is the excessive leucocytosis, at least 150,000 cells, of which a considerable proportion are myelocytes or a still higher proportion are lymphocytes.

No other condition thus far observed yields such characters in the blood in the presence of which the diagnosis of leukemia is established beyond doubt.

2. *The changes in the blood may justify only a probable diagnosis of leukemia.* This situation is encountered under several conditions.

(*a*) In myelocythemia the effect of intercurrent infections may so reduce the number of leucocytes, and especially the proportion of myelocytes, that the blood does not differ from that of some cases of acute inflammatory leucocytosis with 5 to 16 per cent. of myelocytes.

(*b*) In the less marked stages of lymphocythemia, especially of the acute form, the number and proportion of lymphocytes may be far from characteristic of leukemia. When there are less than 150,000 white cells and less than 90 per cent. of lymphocytes the condition of the blood does not differ from that seen in some forms of inflammatory leucocytosis, or of lymphocytosis in the secondary anemia of children, or of the obscure condition called "v. Jaksch's anemia." In inflammatory lymphocytosis, however, there are always a fair proportion of polynuclear leucocytes, which are very scanty in lymphemia, and the lymphocytosis is usually transient.

In secondary anemia the lymphocytosis is seldom excessive, the proportion of lymphocytes is usually lower, and a few myelocytes, eosinophiles, and nucleated red cells are commonly present.

In "v. Jaksch's anemia" the lymphocytes are usually less numerous, there are more large hyaline mononuclear leucocytes than are usually seen in lymphemia of equal chronicity, and myelocytes, eosinophiles, and many nucleated red cells are usually present.

Frankel has classed as acute lymphemia certain obscure cases attended with moderate hyperplasia of lymph nodes and the presence in the blood of a considerable number of cells regarded by some as large lymphocytes. Frankel's cases have not been fully accepted by his countrymen, and Grawitz especially has shown that the above condition of the blood is not pathognomonic of lymphemia, while Benda has paved the way for the division of these cases into other categories, including acute myelocythemia. Frankel's cases may belong in the class of lymphocythemia, but the condition of the blood is not characteristic, and the result of his autopsies is inconclusive.

3. In some stages of leukemia *the blood may fail to furnish indications of the nature of the disease*, which may then be overlooked.

(*a*) Intercurrent infections have been shown to temporarily transform the blood of leukemia into that of inflammatory leucocytosis.

(*b*) In a case of acute myelocythemia, with the disease fully established, the writer found on first examination, 5 per cent. of myelocytes with a leucocytosis of ordinary inflammatory grade. Later the myelocytes increased to the lower limits of leukemia, but a positive diagnosis was not established until the marrow was examined microscopically.

(*c*) Most writers agree that the early stages of leukemia often escape detection, and that many spurious cases have appeared in the literature.

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CHAPTER IX.

PSEUDOLEUKEMIA.

Definition. Pseudoleukemia is a primary disease of the lymph nodes and lymphatic structures, characterized by progressive enlargement of various chains of lymph nodes, and by secondary multiple growths of lymphoid tissue throughout the lymphatic system. It is of toxic and usually of infectious origin, and its essential feature is an extreme hyperplasia of lymphocytes, which approaches or reaches the grade of a neoplasm.

Historical. The first attempt to demonstrate the specific character of the lymphadenopathy of the disease appeared in 1828, when Craigie called attention to the difference between certain firm tumors of lymph nodes and the caseating, scrofulous, and the cancerous enlargements. In 1832 Hodgkin described several cases observed chiefly at Guy's Hospital, some of which were cancerous, tuberculous, or syphilitic, but two of which were undoubtedly genuine examples of pseudoleukemia. He distinguished the "organized" nodes from the caseous and cancerous ones.

Before the discovery of leukemia, therefore, it was known that there are enlargements of the lymph nodes apart from cancer and tuberculosis. In 1839 Velpeau drew attention to the occurrence of hypertrophied lymph nodes apart from scrofula. In 1856 Wilkes described several cases, which he regarded as a special form of disease of lymph nodes, but did not fully distinguish the lesions from those of tuberculosis.

The first complete description of the malady appears to have been that of Wunderlich, 1858, who noted an idiopathic origin of the disease, and the cellular character of the new-growths in both nodes and spleen, mentioned the severe anemia, and called attention to the absence of the extreme leucocytosis which characterizes leukemia. In the same year, 1858, Billroth described the histological structure of the enlarged nodes, noting the limitation within the capsule, the obliteration of follicles and of lymph sinuses and vessels, the proliferation of cells by nuclear division, and concluding that the hyperplasia is closely related to and may pass into sarcomatosis. Recognizing in the condition a tumor of lymph nodes of fatal tendencies, but differing from sarcoma, he employed the term "malignant lymphoma" as specially applicable to this condition. In 1864 the general pathological features were described by Virchow, under the term "lymphosarcoma." Cohnheim in 1867 described a case, and, noting especially the absence of leucocytosis, suggested the term "pseudoleukemia."

The nearly constant affection of the spleen was noted by the earlier observers, Wilks, Woillez, Griesinger, Müller, and Strümpell, and the prominence of this symptom in some cases led Griesinger to employ the term "splenic anemia" for such forms of the malady.

In France, Bonfils described a case in 1858, using the designation "*cachexie sans leukemia*," on account of the absence of leucocytosis.

A few other examples of the disease were reported before 1865, when Trousseau described in detail the clinical characters and proposed the term "adenia." French writers from Trousseau to Gilbert have regarded the condition as very closely related to or identical with leukemia, employing the term "*lymphadenie aleukemique*."

In 1870 a full description of the disease, clinical and pathological, with historical notes to date, was published by Murchison and Sanderson. Cornil and Ranvier, in 1867, very fully described the histological structure of the nodes, regarding the process as a true lymphadenoma. In 1887 Ebstein[1] described an acute form of the malady. Later writers have described similar cases, possibly including among them some conditions not related to pseudoleukemia (Banti, Potain, Bruhl).

The peculiar localization of the lesions to different structures has been illustrated in many reported cases. Bonfils and Trousseau claimed that the spleen need not participate at all in the lesion. A special affection of the tonsillar ring was described by Demange, and of the intestinal mucosa by Gilly, and later by many others. A testicular form has been described by Monod and Terillon. A case of Reineberg's indicates that the lesion may be limited to the marrow. Many have regarded the *anemia infantum pseudoleukemica* of v. Jaksch as the splenic form of Hodgkin's disease occurring in an infant (Luzet, Gilbert).

The *dermal type* of the disease has been described by many writers, including Biesiadecki, Kaposi, Hochsinger and Schiff, and Joseph, but appears to have been first described in France by Gillot.

Anatomical Characters. Lymph Nodes. The essential lesion in the disease is an extreme hyperplasia of lymphocytes. In some cases, especially in the early or active febrile stages, there are prominent signs indicating that the hyperplasia is inflammatory, but in other cases the lesions are those of a simple hyperplasia approaching the neoplastic grade, and the inflammatory changes are in the background.

Gowers has described in different nodes of the same case various stages between simple inflammatory and neoplastic hyperplasia of lymphocytes. The essential lesion may, therefore, be *inflammatory hyperplasia* or *lymphadenoma*.

In the typical chronic cases one lymph node in a chain is affected, followed by the other members of the chain, or the entire chain is affected simultaneously. The disease seldom spreads by gradual extensions from one chain to another, but suddenly involves a new chain on the same or opposite sides of the body. Sarcoma of lymph nodes affects the intervening tissues between chains of nodes. For a long period the swollen nodes are retained in their capsules, but late in progressive cases the members of the chain are variously fused and their capsules largely obliterated. This fusion may result either from rupture of the capsules or from periadenitis.

It is regarded by some as an important distinction of Hodgkin's disease that secondary growths arise only in pre-existing lymphoid structures, while in lymphosarcoma the secondary growths are indiscriminate and more diffuse.

Microscopic examination shows the new tissue to be composed of lymphocytes supported by reticular tissue. There are considerable variations in the character, both of the cells and of the reticular tissue. The cells are usually lymphocytes of small and medium size, among which are a few polynuclear leucocytes, exfoliated endothelial cells, and a few small giant cells. Eosinophile cells are frequently but not always found in considerable abundance (Goldman, Kanter). This local eosinophilia is regarded by Dietrich and Fischer as of diagnostic importance. Reed found it in six of eight cases. Its presence is regarded by most pathologists as an argument favoring the belief in a tuberculous origin of such growths, as eosinophile cells are so frequently found in atypical tuberculous lesions. The lymphocytes are either diffusely distributed or the normal follicles may be preserved for a long period. In the early stages or in less active growths the lymph paths are preserved, but later are obliterated. The reticular tissue is frequently less abundant than in the normal node; later a diffuse fibrosis occurs and the proportion of cells diminishes. The varying proportions of cells and connective tissue yield soft and cellular or hard and fibrous growths. The latter is probably a later stage of the former condition, but all stages are commonly seen in different nodes of the same subject, and dense nodes are said to have been replaced by softer ones (Gowers). There is little tendency in these structures toward caseation, or suppuration, or hyaline changes of chronic tuberculosis.

The lymph nodes of pseudoleukemia may not differ in microscopic structure from those of lymphatic leukemia in any known particular. Nevertheless, they commonly show more of the signs of chronic inflammation and less of the distinctly neoplastic features. In myelocytic leukemia the lymph nodes commonly show a diffuse growth of larger mononuclear cells. According to Birch-Hirschfeld[1] the nodes of leukemia can be injected through afferent vessels and the fluid will pass through into the efferent vessels, while in pseudoleukemia the injection is imperfect and fluid fails to pass through. Attempts to demonstrate lymph vessels about pseudoleukemic nodes have often failed (Billroth), but sometimes they are found distended with lymph. The occlusion of lymph paths may prevent the afflux into the general circulation of cells from lymph nodes, but it still remains an obscure fact that the very abundant and unusually diffuse lymphoid deposits in the viscera fail to cause lymphocytosis.

Relation to Lymphosarcoma. In many cases reported as pseudoleukemia, especially of more acute type, the lymph nodes in the group are fused together, capsules and surrounding structures are infiltrated and destroyed, different chains are united by a continuous growth, and the process has all the gross characters of a malignant tumor. Such nodes show no traces of sinuses or follicles, but are composed of a diffuse growth of cells usually larger than lymphocytes, often containing giant cells and sometimes masses of fusiform cells. In some cases with more or less involvement of pre-existing lymph nodes multiple secondary growths of very wide distribution occur in situations where distinct collections of lymphoid cells do not normally exist. Thus the serous membranes, pleura, and peritoneum may be found studded with myriads of fine nodules of lymphoid tissue. In such cases the local original growth may or may not exhibit distinct sarcomatous characters.

There appear to be all transitional forms between the chronic lymphomata and the rapidly growing, infiltrating, giant-celled lymphosarcomata. In at least one case it has been possible to observe the transformation of pseudoleukemia into sarcoma of lymph nodes (Eisenmenger). Moreover, of the cases of pseudoleukemia which have passed into leukemia, some have shown the histological structure of giant-celled lymphosarcoma. There remains as a separate class of sarcoma of lymph nodes the spindle-celled sarcomata or endotheliomata, which arise from the reticular stroma.

Two views are possible regarding the classification of such growths. One may enlarge the scope of the term pseudoleukemia to include lymphosarcoma, or may throw out such cases of lymphosarcoma, and limit the application of the term to the lymphadenomata.

In the present state of our knowledge it seems unwise to rob the terms Hodgkin's disease and pseudoleukemia of their specific meaning by including with the cases which resemble lymphatic leukemia those which possess all the characters of malignant tumors. It is admissible and convenient to limit the term pseudoleukemia, as is commonly done, to cases exhibiting the following characteristics: general similarity to lymphemia without leucocytosis, considerable involvement of various chains of lymph nodes, in which the separate nodes long retain their identity, absence of involvement of intervening tissue between affected chains, development of secondary growths principally in pre-existing lymphoid structures, histological structure indicating slight malignancy of an inflammatory origin.

Spleen. The spleen is always enlarged, usually to a very considerable size. Microscopically the lesion is found to consist in hyperplasia of the lymphocytes in the Malpighian bodies with more or less obliteration of their outlines, in some increase in the cells of the pulp cords, and in hyperplasia and exfoliation of the endothelial cells resulting from chronic congestion and inflammation. While in some instances the enlargement of the Malpighian bodies produces multiple tumor-like growths, more often these bodies are but slightly enlarged, and are not prominent. The early cellular stages of the splenic lesion may later be replaced by a diffuse deposit of fine fibrils of connective tissue running out from the Malpighian bodies, and involving the pulp cords.

Marrow. The hyperplasia of the lymphocytes in the marrow is usually not marked. The changes usually consist in moderate excess of lymphocytes, swelling, hyperplasia, and exfoliation of endothelial cells, and corresponding loss of the normal cells of the marrow. In some cases there are added the changes of secondary pernicious anemia.

Acute Pseudoleukemia. Various acute and fatal processes of somewhat uncertain nature have been classed with pseudoleukemia.

1. That pseudoleukemia may run an acute course appears from the reports of Cohnheim, Eberth, and Falkenthal, in which the disease lasted from eleven days to four and one-half months, and in which microscopic examination showed the presence of lymphadenomatous hyperplasia of the nodes and metastatic growths in the viscera. These cases occurred in children under fifteen years.

2. Chronic pseudoleukemia may suddenly assume a grave character and prove rapidly fatal from the ulceration of enlarged lymph follicles in the intestines, as first shown by Berthenson's cases, and now not infrequently verified at the autopsy table. Usually such fulminant features in the disease are seen in the early stages of cases, which later subside and pursue a chronic course; or in latent processes which have long existed without attracting attention; while in other cases the acute course is referable to secondary infections grafted on the original process.

3. Acute lymphosarcoma may run a rapidly fatal course and lead to changes in the viscera, which are very similar to those of pseudoleukemia. Fagge collected several cases to illustrate this fact, but their real nature is not always apparent, and the microscopic reports were meagre. They were all attended with purpuric symptoms. As already stated, these cases cannot properly be included in the class of pseudoleukemia.

The most rapid case of lymphosarcoma that the writer has seen lasted four months. The nodes showed a diffuse growth of mononuclear cells much larger than lymphocytes and a few giant cells. There was prompt recurrence of the tumor *in loco*, rapid metastases, marked anemia, and no leucocytosis. Much more rapidly fatal cases have been observed, while Birch-Hirschfeld's case of Hodgkin's disease following typhoid fever was fatal in six weeks.

Relation of Pseudoleukemia to Leukemia. The similarity of the process in the lymphoid tissues in pseudoleukemia and leukemia, is strongly indicated by the histological features already described. The process in pseudoleukemia differs from that in lymphemia in its constant origin in the lymph nodes, whereas lymphemia originates in or constantly involves the marrow. In pseudoleukemia the lymph paths are occluded and the growth is more circumscribed, although usually producing larger tumors, while the signs of an inflammatory process are often distinct in one, while commonly lacking in the other.

Pseudoleukemia may lead to the same changes in the marrow as are seen in lymphatic leukemia (Perrin, Schulz, Dyrenfuth, Kelch, Ponfick,[1] Schmuziger, Baumgarten).

The transformation of pseudoleukemia into leukemia has been reported in several cases Most of these on examination prove to be of uncertain nature, or were undoubtedly instances of terminal or moderate temporary leucocytosis in the course of some disease of the lymph nodes.

Mosler reports an acute case in a child, aged fourteen years, whose blood a few weeks after the onset of the disease showed no leucocytosis, while about three weeks before death the white cells were as numerous as the red. The varieties of cells were not stated. Senator has reported a case of transformation of v. Jaksch's anemia into leukemia. The case of Fleischer and Penzoldt is the most significant. Their patient was a male, aged forty years, who suffered from enlargement of many lymph nodes for sixteen months before death. Four months after the beginning of the illness there was no leucocytosis, but after twelve months there was one lymphocyte to nine red cells. The spleen was moderately enlarged, the lymph nodes extremely large; there was diffuse lymphoid infiltration of the liver, but the marrow of the femur was normal. The microscopic structure of the nodes was not reported, while the absence of a characteristic lesion in the marrow speaks against leukemia. Wende reports a case of pseudoleukemia beginning with multiple tumors of skin and lymph nodes with terminal lymphocytosis of 45,000 cells, 96 per cent of lymphocytes appearing after the fourth month of the disease and one month before death. The day before death the leucocytes fell to 600. The marrow was unchanged.

It thus appears that the statement that pseudoleukemia may pass into leukemia rests upon rather uncertain observations. The more recent case of Posselt is an apparently genuine example of the same transformation, but the clinical history is meagre. The remarkable case of Lucke's of lymphosarcoma with rupture into a vein and development of lymphatic leukemia is also of interest in this connection. One must, therefore, accept with considerable reserve the opinion that some forms of pseudoleukemia represent an aleukemic preliminary stage of leukemia. The vast majority of cases of pseudoleukemia pursue a chronic course showing no tendency to develop leukemia, and recent experience does not support Rothe's belief, that early pleurisy and pneumonia carry off many cases of pseudoleukemia before the leukemic stage has had time to appear. Eisenmenger reports a case of pseudoleukemia which, after pursuing the ordinary course for four years, developed malignant characters infiltrating surrounding tissues and perforating the larynx. The presence of 90,000 leucocytes in this case would have led many observers to suggest the presence of lymphatic leukemia as well.

Relation of Pseudoleukemia to Pernicious Anemia. Runeberg reported a case showing pernicious anemia, which he regarded as an example of purely myelogenous variety of pseudoleukemia, on account of the lymphoid changes in the marrow. The transformation of pernicious anemia into pseudoleukemia was claimed to have occurred in a case described by Laache, and there can be no doubt that the marrow in some cases of pernicious anemia greatly resembles that of pseudoleukemia, as first noted by Scheby-Buch and Pepper. On evidence of this sort, Sevestre suggested that all cases of pernicious anemia fall in the class of pseudoleukemia, but this view cannot be supported on either clinical or anatomical grounds.

Relation of Pseudoleukemia to Tuberculosis. That cases of tuberculous lymphadenitis cannot always be distinguished clinically from pseudoleukemia was early recognized, and so commonly observed that the scope of the disease was by some widened to include such cases. Many of the cases of so-called chronic intermittent fever with swelling of lymph nodes described by Pel, Ebstein,[1] Renvers, and many others, probably belong in this class, as shown by Combemale and Musser.

The exact significance of these cases appears not to have been suspected until Askanazy, in 1888, demonstrated tubercle bacilli in the swollen nodes of such a case. In most of the cases in which microscopic examination of the nodes was reported, there were distinct evidences of tuberculous inflammation, in the presence of miliary tubercles, or caseous foci, or at least abundant hyaline degeneration, and often there was a general tuberculosis (Delafield), but Waetzoldt's case furnished some nodes, showing pure lymphoid hyperplasia, with only very scanty and minute foci of hyaline material, *although containing many tubercle bacilli in sections.* Finally, Brentano and Tangl have described a chronic case in which, with tuberculous lesions in other regions, the lymph nodes showed no recognizable evidences of tuberculosis, not even minute hyaline areas, nor bacilli in section. *Inoculation experiments, however, demonstrated the tuberculous nature of the process.* Numerous observers have recently reported similar cases, while Sternberg demonstrated the tuberculous nature of fifteen of eighteen routine cases.

On this evidence, of which further confirmation appears desirable, it is necessary to admit either that tuberculosis of lymph nodes may follow the most typical course of acute or chronic pseudoleukemia or that tuberculosis and pseudoleukemia may coexist. It is evident, also that microscopic examination of lymph nodes is not a sufficient test of the tuberculous nature of lymphomata, and that the entire subject requires readjustment on the lines indicated above.

That all cases of pseudoleukemia are not tuberculous, however, is indicated by the negative results of inoculation obtained by Westphal, Sciola and Carta, Fischer, Reed, and others, but it must be remembered that the inoculation of moderate quantities of tuberculous material in guinea-pigs is not always followed by tuberculosis.

Miscellaneous Infectious Origin of Pseudoleukemia. Acute or chronic, non-tuberculous, *inflammatory hyperplasia* of lymph nodes may reach such extreme degree as to resemble the condition found in pseudoleukemia. The reported cases of this character are numerous, and have arisen from a great variety of infections, as from carious teeth (Ebstein,[2] Stengel), quinsy (Ponfick[2]), ulcerative pharyngitis (Chvostek), typhoid fever (Birch-Hirschfeld[2]). Other cases have been referred to otitis media, chancroid, eczema, etc. The writer has seen more extensive lymphoid hyperplasia in the intestine in typhoid fever than existed in two cases of pseudoleukemia dying with intestinal ulcerations.

Microscopic examination, where reported, has shown that the hyperplasia of nodes is inflammatory in character and not lymphomatous, as in the cases of Lannois and Courmont, while metastatic growths are wanting. Many of these cases have resembled *purpura hemorrhagica*, as pointed out by Kossler and regarded by him as evidence of infectious character. Lannois, Courmont, and Guillermet isolated pyogenic cocci in their cases.

The occurrence of these febrile forms of the disease and its frequent development after many forms of suppurative inflammation, especially of the mucous membranes, early suggested the idea that pseudoleukemia is, in all instances, of infectious origin. This view was reached by Westphal, after a full review of the subject in 1893, and has since been maintained by Barbier, Verdelli, and others. Verdelli collected fifteen cases of pseudoleukemia, in which the presence of pyogenic organisms was demonstrated in the blood or lymph nodes, viz., *staphylococcus pyogenes aureus*, seven times; *streptococcus pyogenes*, three times; *pneumococcus* of Fraenkel, once; unidentified cocci or bacilli, four times.

Barbier refers the failure of suppuration in these cases and the negative results of some inoculations to a diminished virulence of the germ or to relative insusceptibility of the individual. The accumulating evidence points to the possibility of separating from true pseudoleukemia a considerable group of inflammatory hyperplasias of bacterial origin. At the same time it must be admitted that the micro-organisms in some of the above cases were probably the cause of secondary infections only, and there are opposed to these positive results of culture a number of negative reports.

That *tertiary syphilis* of the lymph nodes may occasionally pursue a clinical course indistinguishable from that of chronic pseudoleukemia has long been accepted by surgeons, and the existence of a syphilitic element in some cases which yielded to mercury has been pointed out by Lannois and Lemoine and others. Lowenbach's study of these cases indicates that the lesion closely resembles that of the tuberculous variety, with rather greater tendency to fibrosis. With few exceptions the syphilitic lymphomata have been of moderate size.

It may be added that Löwit claims to have found the *hemameba leukemiæ magna* in a case of pseudoleukemia.

Summary of Etiology and Pathogenesis. It thus appears that the term pseudoleukemia applies to a group of cases showing chronic enlargement of the lymph nodes usually originated by or associated with an infectious agent, but in which the histological examination reveals a multiplication of cells, apparently beyond the limits of inflammatory hyperplasia, in some cases developing into lymphosarcoma, and possibly in rare instances into lymphemia. That the clinical course of the disease may be perfectly simulated by tuberculosis, and that the more or less specific histological changes in the nodes are in the majority of cases induced by the tubercle bacillus, there is no longer good reason to doubt.

Such overgrowth of lymphocytes in pronounced degree is exhibited in some other manifestations of tuberculosis, so that no new character of this micro-organism is required to account for its connection with Hodgkin's disease.

It seems almost equally certain that other micro-organisms, the common pyogenic bacteria, and syphilis may give rise to the same lesions and history. The essential feature appears to be not a particular micro-organism, but a particular clinical course and histological lesion. This lesion is an extreme hyperplasia of lymphocytes, which lies in the well-recognized border-land between inflammatory and neoplastic processes. In most cases the inflammatory features are distinct, and a tendency to fibrosis is evident. In othgrade of hyperplasia progresses until it seems to fall within the group of neoplasms. That the lymphatic hyperplasia of typical chronic Hodgkin's disease ever does fall distinctly into the group of true neoplasms may still be doubted.

It is possible that the advent of a true neoplastic process affecting lymphocytes is revealed at once by the clinical features of lymphosarcoma or lymphemia, in which case Hodgkin's disease is resolved into lymphosarcoma, on the one hand, and inflammatory hyperplasia, usually tuberculous, on the other. An essential feature, as pointed out by Grawitz, is the general systemic involvement giving rise to multiple lesions throughout the lymphatic system. It also appears that the peculiar clinical condition is not, as claimed by Grawitz, without a peculiar pathological basis, but is, on the other hand, characterized by a most striking cellular reaction, which has numerous counterparts within the domain of cellular pathology. It may even be claimed that the histological process is more distinctly limitable than is the clinical picture, especially in the cases of lymphosarcoma, which may resemble pseudoleukemia at a time when the histology of the nodes clearly indicates a malignant process.

It remains to consider the significance of those cases in which distinct tuberculous lesions are added to the lymphatic hyperplasia. Of these it may be said that the essential feature of pseudoleukemia is present, viz., the excessive hyperplasia of lymphocytes, and that the mere additional presence of the ordinary lesions of tuberculosis is insufficient ground for eliminating these cases from the category of pseudoleukemia, so long as they exhibit the usual clinical and anatomical features of the disease. Nor is it necessary to suppose that tuberculosis is here grafted upon some other process. Usually in such cases, the ordinary tuberculous lesions, if present at all, are coextensive with the simple hyperplastic changes, which is very unlikely to follow a secondary infection by tuberculosis.

The existence of numerous transitional forms between frank tuberculosis of lymph nodes and pseudoleukemia, between pseudoleukemia and lymphosarcoma, and between pseudoleukemia and lymphemia, as well as the increasing number of observations of the coexistence of tuberculosis and lymphemia, suggest that all of these conditions may at times have a common origin.

The Changes in the Blood.

Red Cells. It is a uniform observation that in the early stages of pseudoleukemia the anemia may be very slight. The red cells frequently number 5,000,000 or more when the nodes are distinctly swollen. With the progress of the lesion there is usually a progressive loss of red cells, which, however, is less marked than in corresponding stages of leukemia. In fatal cases there may be surprisingly little anemia, usually the cells fall below 3,000,000, and occasionally the condition of the blood resembles secondary pernicious anemia. In acute cases the anemia may rapidly increase, and the disease resembles a septic infection or a malignant new-growth.

In *morphology* the red cells usually show the changes of simple secondary anemia of chronic course. Laache has called attention to the very uniform size of the red cells in the average case. In some of the writer's cases the cells have been very uniformly undersized. Later megalocytes deficient in Hb may appear, but they are seldom numerous, and do not lead to confusion with pernicious anemia. Laache also reports an obscure case regarded as showing the transformation of pernicious anemia into pseudoleukemia.

Nucleated red cells are usually very scarce, even in late stages, and when present are usually of moderate dimensions. Jawein reports an obscure case of splenic type and afebrile course, in which there were 3840 to 5914 normoblasts. The condition of the blood resembled that of v. Jaksch's anemia, but the patient was an adult.

The Hb is in all cases diminished. A low Hb-index is commonly seen in early stages or with slight diminution of red cells, while in advanced cases with marked reduction of red cells the Hb-index is usually higher. These characteristics are common to most secondary anemias.

The anemia of the typical case of Hodgkin's disease stands in an intermediate position between simple secondary and pernicious anemia. It is usually much less marked than in corresponding stages of leukemia, and almost never approaches the type of pernicious anemia. On the other hand, it is usually more marked than the general condition of the patient would lead one to suspect, thus differing from most forms of secondary anemia. As Grawitz has pointed out, the character of the blood changes offers little encouragement of the tendency to find in this disease a condition related to either pernicious anemia or leukemia.

Leucocytes. The number of leucocytes in the blood is considerably influenced by the character of the process in the lymph nodes. In the majority of cases the leucocytes are normal or diminished in number, and there is a tendency toward relative lymphocytosis. In many cases, however, the leucocytes are continuously increased, with periods of well-marked leucocytosis. The increase of white cells may be considerable, but does not pass beyond the limits of inflammatory leucocytosis, 50,000 to 60,000. Exceptions to this rule must be allowed in the cases of pseudoleukemia which appear to have passed into leukemia, while in Eisenmenger's case, which terminated with sarcoma, there were 90,000 leucocytes. A few eosinophiles are commonly seen in afebrile cases, while an occasional myelocyte has sometimes been encountered.

The cases showing few white cells the writer has found to be usually the chronic ones of slow progress and without fever, while a high proportion of polynuclear cells, with or without absolute increase, usually belongs to the more rapid or the febrile cases. Limbeck finds that when the polynuclear cells are increased the nodes usually show inflammatory changes, but when lymphocytosis is found the nodes approach more to the sarcomatous type. In lymphosarcoma, also, not simulating pseudoleukemia, the small lymphocytes may be very numerous. The writer has been unable to find any features in the blood distinguishing between pseudoleukemia and frank chronic tuberculosis of lymph nodes.

Diagnosis of Pseudoleukemia. 1. From myelocytic or pronounced lymphatic leukemia the diagnosis is readily accomplished by the examination of the blood, demonstrating the absence of characteristic leucocytosis.

2. From less pronounced cases of lymphatic leukemia the diagnosis may not in every instance be possible. Usually the excess of leucocytes in pseudoleukemia affects the polynuclear type, but as the histological structure of the nodes approaches sarcoma the lymphocytes may be greatly increased.

3. In tuberculosis of lymph nodes the diagnosis may require microscopic examination of the nodes. If tuberculous, these may show (*a*) distinct tuberculous lesions, miliary tubercles, and cheesy areas; (*b*) small areas of hyaline material associated with demonstrable tubercle bacilli; (*c*) lymphoid hyperplasia, without any trace of distinct inflammatory changes and without bacilli in demonstrable numbers. Such nodes should be tested by inoculation.

4. Non-tuberculous inflammatory hyperplasia of lymph nodes usually causes irregular fever, polynuclear leucocytosis, and suppuration of the nodes. It is probable that some pyogenic infections of lymph nodes may run the course of subacute pseudoleukemia.

Chronic Splenic Anemia. This term is commonly applied to a group of cases characterized by idiopathic enlargement of the spleen and anemia, but without enlargement of the lymph nodes. While the clinical aspects of these cases are rather characteristic, the underlying pathological conditions have been found so diverse or obscure that many deny that any peculiar condition exists to which such a term can be properly applied. This state of affairs is largely owing to the incomplete investigation of the clinical history and the pathological lesion in the spleen. The writer believes that after all recognizable and well-understood conditions have been separated from this group there remains a set of cases of somewhat peculiar clinical characteristics and with somewhat specific lesion in the spleen to which the name chronic splenic anemia may be given.

To reach this conclusion one must separate from the group of cases of large spleen with anemia the following conditions, all of which are clearly distinct from the others.

Syphilis of the spleen. Gummata.
Large round-celled sarcoma of the spleen.
Chronic splenitis of the type of Gaucher (*epithéliome primitive*).
Chronic malarial splenitis.
Splenomegaly of *anemia infantum* (v. Jaksch).
Splenomegaly secondary to cirrhosis of the liver.

Many writers include under splenic anemia the *anemia infantum* of v. Jaksch—a practice which is entirely unwarranted seeing that the histology of the viscera, both liver and spleen, and of the blood is quite different in these conditions.

Others include under splenic anemia the peculiar condition of the spleen described by Gaucher as *epithéliome primitive*, although the splenic lesion indicates positively that this condition must stand by itself.

There remain for consideration the splenomegaly of Banti's disease, that of marasmic infants suffering from chronic catarrhal gastroenteritis, and that of certain cases in which the lesion of the spleen is that of chronic hyperplastic splenitis, but with which there is neither gastroenteritis nor cirrhosis.

The writer has studied the histological lesion in the spleen in each of these three conditions without finding any essential differences. All show phases of chronic hyperplastic splenitis, with variations only in proportion of cells and diffuse connective tissue

While the splenic lesions in these cases do not differ essentially from some stages of the lesion of pseudoleukemia, yet it appears unwise to regard any of these cases as examples of splenic pseudoleukemia, because none of them develop the lesions in the lymph nodes of that disease while the spleen is often larger than that of pseudoleukemia.

The question remains, therefore, to determine the relation between the chronic splenic anemia of adults, young or old, that of Banti's disease, and that of marasmic infants suffering from chronic gastroenteritis.

That all of these conditions are referable to intestinal toxemia has been repeatedly claimed; and it may be that all represent different stages of essentially the same process. The majority of continental writers accept this view.

Until this etiological relation is established and until it is proven that a marasmic infant with splenomegaly may pass through adolescence as a case of chronic splenic anemia and die later with the lesion of Banti's disease, it may be just as well to apply the term of splenic anemia to the supposed intermediate stage of the process.

The typical case of splenic anemia does not suffer from gastroenteritis and seldom gives such a history, while the disease commonly persists for years without developing the cirrhosis of Banti.

A description of the changes in the blood of this particular group of cases is rendered difficult because current reports usually refer to cases of uncertain nature, and include, especially, examples of v. Jaksch's anemia and Banti's disease. In the more limited group described above, the changes in the blood are those of a slowly progressive secondary anemia without leucocytosis and with relative lymphocytosis.

The red cells commonly number from 3,000,000 to 4,000,000, sometimes less, very rarely falling to the grade of pernicious anemia. They usually exhibit slight changes in size and moderate poikilocytosis. Nucleated red cells are rare, and if present are usually of normal size. The Hb-index is low. Leucocytes are usually diminished in numbers, with relative lymphocytosis, and mast-cells are very scanty. The anemia usually continues for many years without aggravation, and most of the patients die of other diseases, although some develop pernicious anemia, and in some instances the condition is probably the forerunner of Banti's disease.

This entire subject, especially in its clinical aspects, has been reviewed by Osler, through whose writings an introduction to the extensive literature may be secured.

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CHAPTER X.

ANEMIA INFANTUM PSEUDOLEUKEMICA. SPLENECTOMY.

ANEMIA INFANTUM (V. JAKSCH).

Historical. In 1889–90 v. Jaksch described a form of infantile anemia clinically resembling leukemia, but failing at autopsy to show the visceral lesions of leukemia.

The disease was said to be characterized by grave anemia, high and persistent leucocytosis, marked enlargement of the spleen, slight swelling of the liver and occasionally of the lymph nodes, and was to be distinguished from leukemia by the disproportionate size of the spleen as compared with that of the liver, by the more moderate leucocytosis, by the more favorable prognosis, and by the absence of leukemic infiltration of the viscera.

Peculiar forms of grave anemia in infants had previously been described by Italian observers, Cardarelli, Somma and Fede, under the term "infective splenic anemia." Mosler and Senator also had long before recognized and described numerous cases of infantile anemia which they placed in an intermediate position between leukemia and pseudoleukemia.

Shortly after v. Jaksch's first reference to this condition Hayem described a case in which the blood contained numerous nucleated red cells, many showing mitotic figures. The leucocytes were chiefly mononuclear, but the eosinophiles were increased. In Luzet's case nucleated red cells were not very numerous, but eosinophile cells were increased and some mitotic leucocytes were seen.

Thereafter reports of cases multiplied rapidly, so that in 1892 Monti and Berggrun were able to collect sixteen cases, not including those of Hausse and Loos, and added four of their own. In recent years the knowledge of the condition has not been greatly extended, and opinions regarding its significance are still at variance.

Etiology. The typical condition is usually limited to infants and children between one-half and four years of age. The majority of the reported cases have occurred in girls. Rachitis is of undoubted importance in the disease, since it was prominent in sixteen of the twenty cases of Monti and Berggrun. In the cases of severe rachitis reported by Hock and Schlesinger[1] the changes in the blood resembled those of v. Jaksch's anemia, even when the spleen was not enlarged. Syphilis, chronic intestinal catarrh, and chronic tuberculosis were found in other cases, collected by Fischl.

An infectious origin was held for very similar cases as early as 1880 by Cardarelli, and in 1887 by Somma and Fede. Later Mya and Trambusti, Toeplitz, and Gianturco and Pianese, again maintained the infectious nature of the malady. Recently Löwit reports finding the "*hemameba leukemiæ magna*" in one case.

Pathological Anatomy. The changes in the viscera have been described principally by v. Jaksch (Eppinger), Luzet, Baginsky, Audeoud, and Rotch.

The spleen is much enlarged, and usually rather firm. Histologically the changes are those of simple hyperplasia of all elements, while the sinuses contain an excessive number of leucocytes. Luzet found some mitotic normoblasts, but in his cases nucleated red cells were not numerous in the blood. Baginsky found many eosinophile cells in the spleen. Audeoud described extensive proliferative changes in the splenic follicles and pulp, gorging of sinuses with leucocytes, and occasional extravasations of blood.

The liver, in the majority of cases, has been found moderately enlarged, usually less so than the spleen, and of uniform color and normal consistence. Luzet could not verify the claim that the liver is less affected than in leukemia, while in a case of the writer's the liver was quite as large as in fatal cases of leukemia in young children. Histologically there is an absence of leukemic infiltration, but Luzet found between the liver cords a considerable number of large cells (15μ to 25μ in diameter) which he regarded as progenitors of the red blood corpuscles.

In a case of the writer's, examined in 1896, within the liver capillaries there were small collections of nucleated red cells and leucocytes, of which some of both were found in mitotic division, but the characters of leukemic infiltration were entirely wanting. The infiltration with these groups of small cells gave much the same appearance as the late fetal liver. In the fetal liver, however, these foci are composed almost exclusively of nucleated red cells. In one of Rotch's cases also a similar condition was described.

The *lymph nodes* were moderately enlarged in twelve of twenty cases, but in no degree comparable to the changes of leukemia. The *marrow* was described by Luzet as diffusely reddened and moist, and as showing evidence of excessive multiplication of red cells.

The Changes in the Blood.

The red cells are always markedly diminished. v. Jaksch's case, in which they numbered 820,000, showed exceptionally severe anemia, the majority of cases having 1,500,000 to 3,500,000 red cells. The ordinary changes of grave secondary anemia are to be noted in the red cells. Alt and Weiss found poikilocytosis very prominent, but this condition, together with megalocytes and microcytes deficient in Hb, are common to other anemias.

An excessive abundance of nucleated red cells has been shown to be one of the most characteristic features of the blood of this condition, in which they may be even more numerous than in leukemia. Yet they are not always present, in which case the disease becomes difficult to distinguish from pseudoleukemia. Luzet, Alt, and Weiss, and others, have noticed that an unusual number of these cells are found in mitotic division. In well-marked cases the nucleated red cells may all be of normal size for this age, but in the graver stages of anemia megaloblasts reach a considerable proportion or a majority. The usual degenerative changes in the red cells of severe anemia have been described in several of these cases.

The leucocytosis is an important characteristic. Usually the white cells number between 20,000 and 50,000, but in an apparently genuine case of Baginsky they varied between 122,000 and 40,000. In more typical instances the uniform persistence of the leucocytosis without marked variations has been somewhat peculiar. Some cases have recovered with diminishing leucocytosis.

In most instances in which differential estimates are reported the *mononuclear cells* have been slightly in the majority. In apparently genuine cases (Rotch, Hock and Schlesinger[2]) the mononuclear cells, large and small, formed 80 per cent., 84 per cent., and 75 per cent. of a greatly increased number of leucocytes (in one case 116,000). In other cases the *polynuclear cells* have been the more numerous. The proportion of *eosinophile cells* varies. Although considerably increased, up to 6 per cent. (Vickery), they do not reach either the proportions or numbers seen in leukemia. *Myelocytes* have been noted by Klein and in the more recent reports, but only in small numbers. In one of Vickery's cases 10 per cent. of myelocytes are reported among 22,000 white cells. Usually these cells are not so abundant as to suggest leukemia.

Great variations in the size of the neutrophile leucocytes have been described, but cannot be regarded as a special character. The great variety of degenerative changes seen in all the blood cells, especially in the leucocytes, has been very fully described and depicted by Engel.

Significance of "v. Jaksch's Anemia." The attempt to determine the true nature of the cases described under this term must be guided by the known characteristics of the blood of infants. The more important of these characters are: (*a*) *The relative lymphocytosis;* (*b*) *the more active leucocytosis excited by chemotactic influences;* (*c*) *the tendency to enlargement of the spleen in all chronic anemias of infants;* (*d*) *the hyperemia of the marrow associated with rachitis;* (*e*) *the facility with which blood formation in infants partially regresses toward the embryonal type.*

With these disturbing factors in view, it would appear that some of the reported cases of *anemia pseudoleukemica* could better be classed as *pernicious anemia* by referring the peculiar leucocytosis to the special tendencies of infants' blood. Such a case is that of v. Jaksch, in which there were 820,000 red cells and 54,660 leucocytes of undetermined varieties.

Other cases, like that of Rotch, with 1,311,500 red cells, 116,500 leucocytes, 80 per cent. lymphocytes, enlarged lymph nodes, no autopsy, etc., might perhaps better stand as *lymphatic leukemia* with unusual proportion of nucleated red cells. Yet the present tendency is to accept too uncertain evidence in the diagnosis of lymphatic leukemia, and the writer agrees with Rotch in his classification of this case. One of Senator's cases also showed enlargement of lymph nodes and a ratio of 1 to 10 between leucocytes and red cells. Likewise Vickery's case, with 22,000 leucocytes, 35.8 per cent. lymphocytes, 10 per cent. myelocytes, and 6.2 per cent. eosinophiles, and no autopsy, may have been an early stage of *myelogenous leukemia*, but myelemia in infants, even if acute, ought to give more than 22,000 leucocytes and 10 per cent. of myelocytes, while if chronic its characters are usually unequivocal. The real nature of these cases must remain obscure in the absence of repeated and very complete examinations of the blood, and microscopic study of the viscera.

Finally, it is possible that many of the cases are to be regarded as *grave anemia with leucocytosis* of peculiar character. This view is supported by the occasional record of recoveries and of gradual transformations into grave anemia with ordinary leucocytosis (Monti, Berggrun).

Nevertheless, in spite of the resemblance of many cases to pernicious anemia, leukemia, or grave anemia with leucocytosis, there appears to be, in the clinical history, in the morphology of the blood, and especially in the condition of the viscera, sufficient ground on which to separate, at least for the present, certain peculiar forms of chronic anemia in children from any of the above conditions.

In the clinical history these signs are, chiefly, the chronic course, the frequent association with rachitis, syphilis, or chronic intestinal catarrh, the pronounced enlargement of the spleen, and the usual absence of distinct features of pernicious anemia, leukemia, or of a cause of leucocytosis. *In the blood* the changes are rarely such as to cause confusion with pernicious anemia, though the condition appears at times to precede pernicious anemia. While the acute cases with many lymphocytes or myelocytes may be indistinguishable from leukemia (one case of Luzet, XXV., having passed slowly into leukemia), in other more numerous instances the peculiar condition of the blood persists unchanged for months and shows no tendency to declare itself as leukemia. In the average case of grave secondary anemia the leucocytosis fails to show the peculiar characters of this condition. The excessive numbers of nucleated red cells and the very active multiplication of these cells and of leucocytes, as indicated by the large proportion of mitotic nuclei, and the abundance of myelocytes and eosinophile cells, or of large lymphocytes, are signs not necessarily connected with grave anemia in children, but here constitute a striking and distinctive blood picture.

Considering the *changes in the viscera*, it is found that while the other features of the disease simulate pernicious anemia or leukemia, there are neither the leukemic infiltrations and hyperplasias nor the excessive iron-content of the liver, nor the megaloblastic changes in the marrow, of pernicious anemia.

The writer is inclined to believe that the peculiar groups of mitotic red cells and leucocytes found in the hepatic capillaries of the single case which he has had opportunity to examine may indicate the essential nature of the condition. The presence of these cells shows that the liver had resumed or retained its fetal function of blood-cell formation. Similar groups of cells resembling leucocytes have been described by Rotch and similarly interpreted, but without mention of Hb-content or mitotic figures in the cells. Luzet also mentioned the presence of peculiar groups of cells in the liver. If these observations can be verified a specific anatomical condition will have been established for v. Jaksch's anemia, viz., the resumption of the liver under the influence of grave anemia or toxemia of its fetal function of developing red and possibly also white blood cells. The conditions would then differ from pernicious anemia in the absence of extreme megaloblastic changes in the marrow, and in the extension, rather, of megaloblastic red-cell formation beyond the marrow and into the next most available tissue, which is the liver. On this anatomical basis it would still be impossible to explain the frequent presence of many lymphocytes or myelocytes which, however, may be referred to the coincident affection of lymphoid tissues, especially of the marrow, a tissue which in this disease has as yet received inadequate attention.

In a case observed by the writer in 1896 the patient was a markedly rachitic infant of twenty months. The spleen extended three inches below the costal border. The liver was considerably enlarged. The lymph nodes were not enlarged. The red cells numbered 1,820,000, about 10 per cent. of which were pale megalocytes. Nucleated red cells were extremely abundant and usually of normal size. Hb not taken. The leucocytes numbered 48,000, of which 22 per cent. were small lymphocytes, 34 per cent. large lymphocytes, 3 per cent. myelocytes, 38 per cent. polynuclears, 3 per cent eosinophiles.

The *spleen* was the seat of uniform hyperplasia of pulp cells, while the follicles were much reduced in size and number. Throughout the pulp there were numerous small collections of small cells with compact or mitotic nuclei. Many of these were nucleated red cells. Mitotic leucocytes were not identified. There was beginning increase of connective tissue and the sinuses were often obliterated. Eosinophile cells were very abundant. The *liver* exhibited numerous small intracapillary foci of cells, many of which contained mitotic nuclei. Of these the majority were nucleated red cells, others were much larger and granular. Occasionally they appeared fused together in one celi mass, resembling the multinuclear masses of Luzet. These groups of cells resembled the foci of nucleated red cells of the embryonal liver, but were much less numerous. The liver cells were not fatty.

The *marrow* of the femoral shaft was cellular throughout, no fat cells being found in this situation, which, in normal subjects of this age is at least partly fatty. There was general hyperplasia of cellular elements, but the cells were not densely packed as in leukemia. The islands of nucleated red cells were very numerous, these cells being distinctly in excess. Many mitotic leucocytes with granular protoplasm were identified. The sinuses were obliterated. Eosinophile cells were not overabundant. The condition of the spleen, liver, and marrow indicated that excessive demands were being made upon the blood-forming organs, and that these demands were being met by the marrow, the liver, and probably also by the spleen.

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SPLENECTOMY.

The effects of splenectomy upon man are usually the combined results of severe hemorrhage, or pre-existing anemia, of the loss of the function of the organ, and very often of intravenous infusion practised immediately after the operation.

How much of these effects are referable solely to the loss of the organ can better be determined by comparing the blood changes following splenectomy in animals with those observed in the human subject.

Effects of Splenectomy in Animals.

Mosler was one of the first to study the effects of splenectomy upon healthy dogs. He found a diminution of red cells, persisting for several months, but no changes in the leucocytes. The blood-forming function, then regarded as inherent in thespleen, he believed to have been transferred to the marrow, in which tissue he found changes comparable to those of leukemia. The chief permanent chemical alteration he found to be a loss of iron. Malassez found the oligocythemia to persist only one month, while the Hb remained deficient much longer. Zesas found in rabbits a marked leucocytosis, reaching a maximum in the tenth week, when the red cells begin to diminish. The blood was restored to the normal six months after the operation. Winogradoff found that the red cells in dogs diminish for 200 days, and many megalocytes appear. In the second year smaller cells reappear, and the total number gradually becomes normal. In two of three dogs there was considerable leucocytosis. Diminution of specific gravity and of Hb was constantly noted. The lymph nodes of the animals were found to be smaller, and the marrow hyperemic. Gibson found the maximum diminution of red cells sixty days after splenectomy (three dogs). Pronounced leucocytosis was observed the day after the operation. The restoration of the blood required six months.

More recently Kourloff followed the course of the leucocytosis of splenectomy, finding in the first year following the operation varying grades of lymphocytosis, the proportion of these cells rising from 30 up to 60 per cent. The proportion of granular leucocytes fell from 40 or 50 per cent. to 20 per cent., or less. The number of large mononuclear cells did not change, indicating that the spleen cannot be considered as their place of origin. During the second year a prominent alteration of the blood was a marked eosinophilia, while in this period the lymphocytes fell to the normal proportion.

Emilianoff demonstrated in splenectomized dogs a slight diminution of red cells and an extreme leucocytosis marked by an initial loss of small cells, and a sudden increase of larger (polynuclear) cells. Vulpius also observed in several rabbits a moderate leucocytosis lasting not longer than nine weeks, and a loss of 20 per cent. in the number of red cells, which were restored within five to six weeks.

It has therefore been shown, by the above observers among others, that splenectomy in animals is followed by moderate reduction in red cells lasting from one to twelve months, by relatively greater loss of Hb more slowly restored, and in some cases by the appearance during the first year of megalocytes.

Leucocytosis follows the operation, but its extent and duration are very variable. A polynuclear leucocytosis is observed during the first days or weeks, followed by relative or absolute lymphocytosis during the first year, while during the second year distinct eosinophilia may be observed.

With these changes in the blood are associated a marked cellular hyperplasia of the marrow, approaching at times that of leukemia, and often also affecting the lymph nodes. In the swollen nodes an excessive number of nucleated red cells have been found by Winogradoff, Tizzoni, Gibson, Kourloff, and Grunberg.

Splenectomy in Man.

The most marked changes in the blood following splenectomy in man are seen in those cases in which the organ has been removed for rupture or idiopathic enlargement. Successful cases are recorded by Czerny,[1] Regnier, Hartley, McBurney, and others. In Czerny's case of idiopathic enlargement (1500 grm.) the Hb fell to 56 per cent., while the red cells were very slightly reduced and reached over 5,000,000 within a month; but four years later the patient, for reasons not stated, was found to have only 3,300,000 red cells with 85 per cent. of Hb. The leucocytosis was very marked, reaching 70,000 within a week and persisting for at least eight weeks.

In Regnier's case of rupture of spleen with severe hemorrhage the Hb fell to 20 per cent., rising gradually to 80 per cent. in the eighth week. The red cells, falling to 2,500,000, rose to 4,700,000 by the eighth week. An acute leucocytosis, 25,000, appeared soon after the operation. One month later the polynuclear cells had been largely replaced by an equal number of lymphocytes. Nucleated red cells and eosinophiles were scarce.

Hartley's case (examined by the writer) was complicated by infusion, and possibly by pre-existing malaria. The anemia on the fourth day was profound; the leucocytosis was extreme, estimated roughly at 75,000 cells, of which 77 per cent. were polynuclear. Many "splenocytes" and a moderate number of myelocytes with deficient neutrophile granules, and a few normoblasts, were present, so that the blood strikingly resembled that of leukemia. At the end of three weeks the young patient's blood had greatly improved, and the leucocytes were normal. After three months the only abnormality was the presence of a few moderate-sized megalocytes and an apparent deficiency of neutrophile granules. Later examinations, extending over three years, failed to show at any time an absolute increase of either lymphocytes or eosinophiles or any persistent anemia.

In McBurney's case the writer found the anemia and the resemblance to leukemic blood even more pronounced. The same prompt improvement followed, and six months later the blood was practically normal.

After splenectomy for various other general indications, not including malaria and leukemia, rather variable results have been noted. In the cases collected by Vulpius, Hartley, and Litten, and reviewed by various authors, the writer finds a general resemblance of the blood changes when reported to those described after experimental splenectomy. The grade of anemia and the period required for the restoration of the blood seem to vary with the general condition of the patient and the circumstances of the operation. Dominici describes a case in a tuberculous subject in whose blood, twelve days after the operation, a very large number of nucleated red cells began to appear, 9800 per c.mm. having been counted at one time. After three weeks they disappeared.

In the majority of the cases of splenectomy for *wandering spleen* and other conditions not seriously affecting the patient's health, the blood was described as normal before and after the operation, but in some the usual reduction of red cells and leucocytosis have been observed. The slight changes normally noted are probably referable to the effects of the laparotomy, as Hartmann and Vaquez found that after every such operation there is slight anemia and leucocytosis.

The *malarial spleen* has been excised by many surgeons with favorable results. Jonnesco, who reports a series of cases, found a prompt increase of red cells after a temporary diminution (500,000 to 2,000,000) and a somewhat persistent leucocytosis (15,000 to 30,000). The operation effected a prompt cure of long-persistent cachexia. Similar favorable results were obtained by Hartley, Vulpius, and others.

In *leukemia* Hagen collected forty-two extirpations of the spleen with four recoveries from the operation, and to these must be added one case of Richardson, reported by Warren, in which the patient recovered while the effect on the blood remained *sub judice*. One case survived thirteen days, another eight months, and a third, in which the diagnosis must be doubted (Franzolini, 1882), was reported cured. In two genuine cases the operation was followed by steady diminution of red cells and increase of leucocytes (Bardenhauer, Burckhardt).

Visceral Changes following Splenectomy. Enlargement of lymph nodes has been observed after splenectomy in man by Czerny,[2] Kocher, Lennander, and Regnier. In Regnier's case the enlargement of the nodes was associated with lymphocytosis. The marrow was examined by Regnier four weeks after the splenectomy and found very hyperemic. There had been marked anemia in this case.

Resume. *In comparatively healthy subjects* splenectomy has often been performed without affecting the blood more than does any other laparotomy.

In many graver cases the loss of blood and the shock of the operation give rise to a considerable grade of secondary anemia. The red cells are, in favorable cases, restored in one to three months, but in less favorable cases there may be more persistent anemia. The restoration of Hb seems in some cases to fall behind the improvement in cells rather more than in most secondary anemias. The operation is usually followed by considerable polynuclear leucocytosis (15,000 to 50,000), which commonly persists for two to six weeks, but may continue for months, in which case the polynuclear cells may be largely replaced by lymphocytes. Eosinophilia has been observed in a few cases during the second and third years.

In traumatic cases suffering from large hemorrhages, splenectomy, especially when complicated by infusion, may lead to very profound anemia, marked by extreme loss of red cells, the presence of many very large, pale, sometimes polychromatic, and dissolving red cells, nucleated red cells, and to a high grade of leucocytosis. Among the leucocytes there may be a considerable proportion of large, pale, mononuclear cells and myelocytes, *so that the blood resembles that of acute leukemia.* This condition, however, is transitory and the blood may improve rapidly. Leukemia and the amyloid spleen are contraindications to splenectomy. In other conditions the choice of operation may depend entirely upon the general condition of the patient. Beyond a moderate persistent leucocytosis or lymphocytosis, and possibly a slight delay in the restoration of Hb, there are no specific effects known to follow splenectomy in man.

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PART III.

THE ACUTE INFECTIOUS DISEASES.

INTRODUCTORY SECTION.

THE BLOOD IN FEVER.

WHILE all observers have agreed that the blood in fever suffers a **reduction in red cells**, it still remains a matter of doubt whether a febrile process alone actually destroys red cells or only causes them to be unequally distributed in the body.

The theory of unequal distribution of red cells in the acute fevers is supported by a variety of observations. Maragliano demonstrated a contraction of arterioles during the height of a febrile process, followed by dilatation during defervescence, and he was able to verify these results by watching the effects of antipyretics. Breitenstein found an excess of red cells in the livers of heated animals coincident with a deficiency in peripheral vessels, while Naunyn under the same conditions could find no evidence of destruction of red cells. Increase in specific gravity of the blood was demonstrated by Stein, with rising temperature, followed by lowering of gravity in defervescence. Reinert believes that excessive loss of fluids diminishes the volume of blood during the height of the fever, while retention of fluids results from lower blood pressure and heart weakness after defervescence. Tumas believes that the blood in fever may be reduced in bulk as well as in proportion of red cells.

In favor of the view that the red cells are destroyed in fever is the demonstration of an excess of potassium (Salkowski) and of hydrobilirubin (Gerhardt, Hoppe-Seyler) in the urine. Yet Bunge has shown that the red cells, which are very rich in potassium, may take up and discharge large amounts of this element without themselves being destroyed. In pure types of experimental pyrexia Naunyn and others have found no solution of Hb in the blood serum and no evidences of destruction of red cells. Werhowsky, however, exposed rabbits to a temperature of 38.5° to 40° C. for two to twenty-nine days and found a steady diminution, reaching 30 per cent. of Hb, followed by progressive loss of red cells, moderate leucocytosis, and deposits of hemosiderin in the marrow and spleen. Similar indications were obtained long ago by Mobitz in septic animals whose blood at first showed from day to day considerable variations in the red cells, but eventually a permanent loss.

These results leave no doubt of the capacity of prolonged fever to destroy red cells, but the length of time required furnishes opportunity for factors other than pyrexia to intervene. Consequently Löwit[1] concludes that the real nature of the oligocythemia in the early febrile process is still doubtful.

Fever is usually accompanied by marked **disturbance of the coagulation** of the blood, which has sometimes been found increased, at others diminished or entirely inhibited. From the studies of Schmidt and his pupils it has been shown that in septic fever coagulation is diminished at an early stage and increased at another later stage. The variations depend, according to Bojanus, upon changes in the quantity either of fibrinoplastic substances or of fibrin ferment, and are connected with the presence of dissolved Hb in the plasma. In non-febrile pyrexia, insolation, the coagulability of the blood is lost.

Changes in Chemical Composition. A progressive loss of albumins of the blood in febrile diseases has been noted by many observers, but it is not yet known whether the loss is referable to the pyrexia or to other associated factors. Rather favorable clinical conditions for determining the effects of fever were found by Stejskal in a case of intermittent Ebstein's disease, in which during the febrile intervals he demonstrated a decrease of albumins and diminution of ash in the whole blood, in the serum, and in the red cells.

While there is good reason to believe that the less diffusible globulin should suffer less than the albumin, as Gottschalk claims to have found in some instances, yet Limbeck and Pick found that no general rule could apply to variations in the globulin of the blood in infectious diseases. A contrary conclusion also was reached by Emmerich and Tsuboi, who found the globulin of the blood to diminish when rabbits are being immunized against hog-cholera.

Resistance of Red Cells in Fever. A diminished resistance of the red cells and increased isotonic tension of the plasma were demonstrated by Maragliano in severe anemia, and by Celli and Guarnieri in various fevers, while the morphological characters which indicate this change in the red cells have been described by Maragliano, Gabritschewsky, and Grawitz. Yet the resistance of the red cells is not found diminished in all fevers and may, as Limbeck[1] has shown, be at times increased. While Hamburger has shown that the resistance of the red cells depends principally upon the osmotic tension of the plasma, Demoor refers the low resistance in fever to the presence in the blood of bacterial toxins and of excess of CO_2, while Löwit[2] connects it more closely with changes in the vessel walls.

Febrile Hydremia. A marked diminution of the albumins of the plasma has been demonstrated in many infectious diseases by Hammarschlag, Limbeck,[2] Biernacki,[1] Wendelstadt, and others, and has been referred not to increase in the total bulk of water in the blood, but to destruction of albumins in the febrile process. Herz believes further that the febrile hydremia is characterized also by swelling of the red cells with corresponding oligoplasmia, and Limbeck and Steindler found the volume of the serum in three healthy subjects to average 72.6 per cent., while in eight acute febrile cases it fell to an average of 54.8 per cent. On the other hand, Pfeiffer denies that the red cells swell in febrile diseases, finding rather that they shrink, and Biernacki denies the existence of any uniform febrile hydremia. The conclusions of each of the above observers appear to be justified by their results, and their conflicting opinions must be referred to technical errors or to particular conditions existing in the subjects of their study.

Alkalescence. Abundant sources of acid metabolic products are believed to exist in fever as a result of imperfect oxidation of albumins and the formation thereby of fatty and lactic acids, as well as in the development of acids as a result of bacterial growth. Numerous observations, also, by Senator, v. Jaksch, Kraus, Klemperer, and others, have apparently shown by different methods that febrile processes are regularly marked by diminished alkalescence of the blood. Yet Minkowski has from the first pointed out that at the height of the fever the loss of alkalescence is not proportional to the pyrexia. Limbeck and Steindler in a large series of febrile subjects found in both blood and serum variations in alkalescence quite within normal limits.

On the other hand, Lowy and Richter, using a special method which they claim to be more reliable than others, find that in a certain period of febrile processes corresponding to the stage of hypoleucocytosis the blood shows an increase and not a decrease of alkalescence. The cause of this condition is not yet explained, but Lowy's results have been verified by Löwit, Biernacki,[2] and Strauss.

Important contributions to the subject were made by Fodor and Rigler. These observers found that the serum of rabbits infected with anthrax at first shows an increase of alkalescence, but after twenty-four hours a rapid and marked diminution. With rabies the alkalescence diminished from the first. Between the quantity of diphtheria toxin injected and the final loss of alkalinity therefrom they found a parallel. Injections of vaccine increased the alkalescence for seven to eight days, of diphtheria antitoxin for only forty-eight hours. Since the alkalinity of the serum-ash did not vary with that of the serum they concluded that the property depends on the presence of organic substances. Other important studies of the alkalescence of the blood by Cantani, Calabrese, and others have shown that the important variations in the quality of the blood are dependent less upon fever than upon other obscure chemical processes.

Lowit[3] *concludes that the alkalescence of the blood may be increased at one time and diminished at another period of an infectious disease, that this property is not dependent in any large measure upon the leucocytes, and that its significance is still unexplained.*

Action of Bacteria and Their Products on the Blood. Many of the phenomena classed among the effects of fever on the blood can be directly traced in part to the action of bacteria and bacterial products which are the exciting causes of the febrile process.

Bouchard has demonstrated for the bodies of *bacillus pyocyaneus*, and Gley and Charrin for their filtered products, a vasoconstricting action, and therefore a tendency to concentrate the blood. An increased flow of lymph and probably a similar concentrating effect upon the blood has been shown to follow the injection into animals of peptone and animal extracts, by Heidenhain; of tuberculin, pyocyanin, pneumotoxin, by Gartner and Romer. Rapid variations in the gravity of the blood have, in Grawitz's[1] hands, followed the injection of cultures of cholera, diphtheria, and pyogenic organisms. Small doses of toxins of various bacteria were found by Bianchi and Mariotti to increase the isotonic tension of the blood quite beyond normal limits, while large doses of many germs or even small injections of *bacillus typhosus* had the opposite effect.

Specific bacterial products, toxalbumins, bacterioproteins, and ptomains are believed by most observers to be the active agent in the destruction of the albumins of blood and tissues in infectious diseases, acting at times without the accompaniment of fever (Müller).

As a specific effect of the chemotactic action of bacteria and their products upon the white blood cells there is the whole series of phenomena of leucocytosis, the significance of which is considered in another section.

Conclusions Regarding the Blood in Fever. From the foregoing brief review it will be seen that the changes in the blood in fever form an extremely complex subject, about which our knowledge is still rather fragmentary. The same phenomena have been encountered in different lines of investigation and attributed to different single factors, though probably referable to many. While some main facts are rather distinctly apparent, other questions must remain undecided until fully adequate technical methods are devised.

Decrease or relative increase in the proportion of *red cells*, but ending always in a loss in their total numbers, must be accepted as accompanying all cases of pyrexia, although requiring some time to become clearly apparent.

Coagulability varies in different stages of febrile diseases, but is not clearly connected with the pyrexia as such.

The progressive *loss of albumin* of the blood is probably essentially connected with the febrile process, but occurs in increased degree when the fever is of infectious origin.

Febrile hydremia is an accidental condition which may or may not occur as a result of the loss of albumins of the blood. *Diminished resistance of red cells* occurs in the majority of fevers, and depends on a variety of factors. *Variations in alkalinity* are frequent and considerable in fever, but are not proportional either to the height of the temperature or to the toxic condition of the blood.

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CHAPTER XI.

PNEUMONIA. DIPHTHERIA.

PNEUMONIA.

The gross characters of the blood in pneumonia were accurately described by Piorry, who noted the prompt and firm clotting due to excess of fibrin, while the distinct *crusta phlogistica*, most marked about the seventh or eighth days, was the earliest recorded observation concerning the leucocytosis of this disease. Fibrin has since been shown to be abundant in nearly all cases of pneumonia, and very much increased in the great majority. The excess often continues beyond defervescence. In very severe cases without leucocytosis, fibrin is usually deficient (Turk[1]).

The total volume of blood is probably somewhat reduced in severe cases of pneumonia, owing to the concentration which occurs in fever, from cyanosis, and from the loss of cells and plasma in the exudate. Evidences of this concentration have accumulated from several sources, among which are: the increase in specific gravity during the height of the process (Monti, Berggrun), and the persistence of a high proportion of red cells and Hb which usually continues until the crisis, after which there is a rapid decline in the quality of the blood.

Large exudations into the lungs are believed by Bollinger to reduce the quantity of blood to such an extent that oligemia may usually be noted at autopsy. In 65 per cent. of his cases marked diminution of blood was thus noted at the postmortem examination, a loss which was fully explained by the extent of the exudate, which averaged over 1000 grm. in weight.

The Red Cells. Although destruction of red cells undoubtedly occurs in the disease, as shown by the increased excretion of hydrobilirubin, and the occasional appearance of jaundice, yet the proportion of red cells remains high during the active febrile period, when this destruction is taking place, to diminish only when the temperature begins to fall. This result can be referred only to the concentrating effects of fever, exudation, and local vasomotor phenomena, and is to be seen in other infectious fevers.

In spite of these complicating factors the red cells usually show a slight but steady decline during the course of the fever, as shown by the reports of Sorensen, Boeckman, Halla, Tumas, Sadler, and others.

Slight polycythemia (maximum 7,000,000) frequently appears in the reports of Sadler, while Sorensen observed an increase of red cells during the febrile period in one case. The decrease of red cells often occurs suddenly with the crisis, Tumas reporting one case with a fall of 600,000 on the day of defervescence. The diminution continues for a very variable period depending upon somewhat complex factors, but commonly ceases within ten to fourteen days, or in mild cases much sooner.

The grade of anemia established is usually not very marked. In many recorded estimates the loss of cells did not exceed the limit of error. A loss of 500,000 to 1,000,000 is not uncommon, while a reduction of 2,000,000 cells is recorded by several observers. Such changes in the red cells, however, must be interpreted with great caution. There was, for instance, a loss of 2,000,000 cells in one of Sadler's cases, yet no anemia was established, as the blood had been concentrated and the lowest count was 5,100,000.

Morphological changes in the red cells are usually slight. Polychromasia is seen in the severe cases only.

NUCLEATED RED CELLS were present in seven of Turk's eighteen cases. These were usually normoblasts, occasionally megaloblasts. The cases were all very severe, but only one of the seven was fatal. A similar phenomenon may occur in other severe infections.

The Hb suffers considerably more than the red cells, being almost invariably reduced after defervescence, but in the absence of complications seldom falling below 65 per cent.

Chemistry. The specific gravity was found to remain normal or to distinctly increase during the febrile period in nine children studied by Monti and Berggrun.[1] The albumins of the blood were slightly diminished, while those of the serum were normal or increased in seven cases reported by v. Jaksch.[1]

The *toxicity of the blood serum* was found much increased in pneumonia and other infectious diseases by Albu, who locates the poisonous principle in the albumins of the serum.

Leucocytes. Leucocytosis appears in the great majority of cases of pneumonia, failing only in very mild attacks and in very severe infections with feeble reaction and bad prognosis, and when some pre-existing conditions have already excited leucocytosis or debilitated the system. In leukemia a complicating pneumonia reduces the leucocytes, while the onset of pneumonia in the course of some infectious diseases is not always traceable in the blood.

Although the leucocytosis of pneumonia was noted by Virchow, Nasse, and other early writers, its closer study was begun by Sorensen in 1876, and continued by Boeckman, Halla, Tumas, Hayem, and Limbeck.[1]

Even Boeckman (1881) gathered from the literature that most febrile diseases are accompanied by leucocytosis, and that typhoid fever and malaria are exceptions to the rule. Halla noted that the leucocytosis is not proportional to the fever, having encountered three cases without leucocytosis, all fatal. Hayem and Limbeck followed the course of the leucocytosis over considerable periods, pointed out relations between the grade of leucocytosis and the severity of the disease, discussed the time and manner of its disappearance, and reported other fatal cases without leucocytosis.

Limbeck's study was especially minute and he was able to detect a fall in the leucocytosis some hours before the crisis, and to note the absence of any change during pseudocrises. He drew the important general deduction that leucocytosis precedes and is a part of the inflammatory exudate, and that non-exudative diseases are unaccompanied by leucocytosis. Rieder, in 1892, was probably the first to demonstrate that the leucocytosis of pneumonia is not determined by the height of the fever or the extent of the exudate, but depends upon the intensity of the infection and the degree of resistance of the subject, and this view was fully supported shortly afterward in the writer's series of cases.

Since Limbeck's study the leucocytosis of pneumonia has been a favorite field of investigation, so that an extensive literature in many languages has accumulated, and many interesting and important details have been added to the knowledge of the subject.

Course of the Leucocytosis. Leucocytosis appears very early in the course of pneumonia, simultaneously with the chill, according to Klein; preceding the exudation, according to Limbeck, and having repeatedly been found on the first day of the disease. In one of the writer's cases 25,000 cells were counted within four hours after the beginning of the first symptom, a sharp chill, while Rieder and Laehr found a marked increase within six hours of the chill. Theoretically the leucocytosis should be preceded by a period of hypoleucocytosis, but this period has never been observed clinically, except in fatal cases with prolonged hypoleucocytosis.

The *maximum increase* is reached usually just before the crisis (Hayem, Klein, Bieganski), but has been observed on the first day of the disease or on succeeding days. When the leucocytes increase slowly they usually diminish slowly, and the disease defervesces by lysis. Peculiar cases of marked severity in which there was no distinct leucocytosis until the temperature began to fall are reported by Bieganski and Turk.

During the high febrile movement there is usually little alteration in the leucocytosis, but extension of the lesion to other lobes, or to adjoining serous membranes, may cause irregular rises in the count. Yet in a case in which bronchial breathing passed successively up one side of the chest and down the other, the writer found rather uniform and high leucocytosis. In six cases Lambert found the leucocytes doubled in numbers with the advent of empyema, which sometimes failed to raise the temperature or give physical signs. Empyema caused a greater increase of leucocytes than did extension of the lesion to new lobes, or resolution.

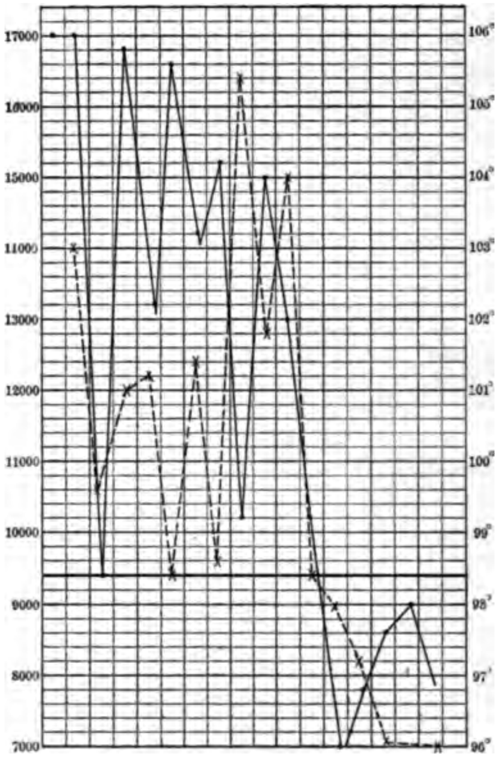
A few hours before or after crisis the leucocytes begin to diminish rapidly, sometimes falling from a high to a normal figure within twenty-four hours, after which there are commonly some slight oscillations. During lysis the reduction of leucocytes usually keeps pace with the temperature, but Limbeck's, Laehr's, and Billings'[1] charts show some marked upward curves of the leucocytes after lysis. Such rises are most often due to complications (Kuhnau[1]). Several of Lambert's cases showed very pronounced increase of leucocytes (maximum 100 per cent.) as resolution began.

In *fatal cases* there is often a continuous increase (Pick), but sometimes the leucocytes are high at first, but steadily diminish as the patient grows worse (Bieganski, Rieder), while in many fatal cases there is no leucocytosis. Hayem believed that in fatal cases without leucocytosis the lesion is "less exudative," but subsequent reports have shown this view to be inapplicable to the majority of cases.

Alcoholism, virulent infection, and old age are more important factors. In four such cases of the writer the lesion was of the usual type. In one of these, although the exudate was considerable, the marrow of the ribs and vertebræ failed to show the usual hyperplasia.

Pseudocrises usually have no effect upon the leucocytosis (Limbeck, Klein, Laehr, Bieganski), but Turk and others have shown that this is not an invariable rule.

FIG. 33.



——Temperature. ------Leucocytosis.
Course of leucocytosis in pneumonia. (After Laehr.)

The grade of leucocytosis is usually considerable, and is affected by several factors. Hayem found in mild cases 6000 to 12,000 leucocytes, in moderately severe cases 18,000 to 20,000, and in very severe cases a maximum of 36,000. *In children* the increase is usually greater than in adults (Monti, Berggrun, Felsenthal). Severe uncomplicated cases often show 50,000 cells, while some writers have found purulent complications with many of the high leucocytoses, especially when the temperature is relatively low (Smith). The highest figures appear to be 115,000, recorded by Laehr, in a peculiar case with delayed resolution. The *fever* and the leucocytosis often run parallel, but there are numerous individual exceptions to this general rule, the most significant of which are the fatal cases, showing normal or diminished leucocytes. The *extent of the exudate* has considerably more influence upon the leucocytes, as first shown by Limbeck. Although contrary conclusions have been reached by many writers reviewing short series of cases, it nevertheless remains true that the leucocytosis in general bears a rather close relation to the extent of the exudate. In the writer's cases, especially in those which came to autopsy, this rule was readily verified, although its application to individual cases proved unsafe. Thus, in sixty-three cases in which one lobe was affected the average number of leucocytes was 20,000; in twenty-four cases with two lobes involved, 22,700; in twelve cases with three lobes, 25,000; in one case with four lobes, 27,000, and in one in which there was bronchial breathing over the entire back of chest, 32,000. In ten cases with lesions extending to the pleura (empyema), pericardium, and peritoneum the average was 17,000—a tendency toward hypoleucocytosis beginning to appear.

The *degree of systemic reaction to the disease* chiefly determines the grade of leucocytosis. This view, first clearly stated by Rieder, has been fully verified by many observers, and embodies the true meaning of leucocytosis in infectious diseases. In forty-seven cases marked by vigorous systemic reaction (temperature 105°, full pulse, sthenic condition), the writer found an average of 30,000 leucocytes; in twenty-seven moderate cases (temperature below 105°, symptoms less severe), the average was 20,000; in twenty-seven cases with deficient reaction, 9000, while in twelve asthenic cases the leucocytes were subnormal.

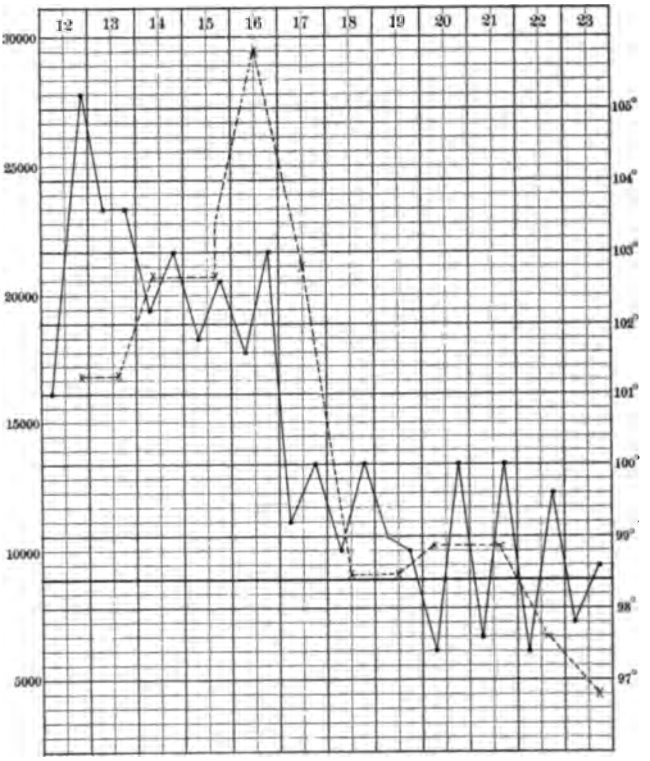
The significance of hypoleucocytosis, observed in many fatal cases, has been demonstrated in various experimental infections like those studied by Tschistowitch, who found that rabbits die without leucocytosis when inoculated with virulent cultures of the pneumococcus, but with attenuated cultures there is leucocytosis and the animals usually recover.

Types of Leucocytes in Pneumonia. At the height of nearly all well-marked leucocytoses in pneumonia the polynuclear neutrophile cells form 80 to 95 per cent. of the cells. Turk counted as high as 96.5 per cent. of these cells, and they are frequently above 90 per cent. This high proportion may be seen when the leucocytosis is slight or absent, but is usually most marked when the white cells are very numerous. At the same time there is a marked reduction, relative or often absolute, of lymphocytes to 2 to 4 per cent. The large mononuclear cells usually persist in considerable numbers (Turk), may sometimes be distinctly increased (Klein), and are never entirely absent (Jez).

The eosinophile cells at the height of the leucocytosis are always very scanty and frequently cannot be found after very prolonged search (Zappert, Turk). Becker, being unable to find any eosinophile cells in fatal cases, concluded that their complete disappearance is an unfavorable sign.

Instead of polynuclear leucocytosis there may be well-marked lymphocytosis, as in a case reported by Cabot, in which in a child aged six years there were 66 per cent. of lymphocytes among 94,600 white cells. Stienon refers to similar cases, and the writer and others have observed such inflammatory lymphocytoses in diphtheria. During defervescence the polynuclear cells diminish rapidly, usually to a high normal proportion, at which they are apt to remain several days. The lymphocytes at the same time increase in proportion, and often in numbers, reaching their normal figures also after a few days.

FIG. 34.



——Temperature. ---------Leucocytosis.
Precritical leucocytosis in pneumonia. (After Limbeck.)

The large mononuclear leucocytes are usually overabundant at this stage, reaching 16 per cent. in one of Turk's cases, and constituting the "large-celled lymphocytosis" described by Klein at this period. Eosinophile cells usually reappear in scanty numbers on the day before defervescence, or rarely a day earlier (Turk). Distinct post-critical eosinophilia occurs in a moderate proportion of cases (Zappert, Bieganski), but not in all (Turk).

In severe cases without leucocytosis the polynuclear cells are usually relatively increased (Rieder), but may be normal (Billings).

Very often in such cases there is a moderate leucocytosis during or after defervescence (Laehr).

Degenerative Changes in Leucocytes in Pneumonia. Klein described numerous "leucocyte shadows" in severe pneumonia. They are seen in other infectious diseases, especially diphtheria, and are to be classed with the degenerative changes in tissue cells which mark the *status infectiosus*. *Glycogen granules* in the leucocytes have been found in considerable abundance by Livierato and others, usually in proportion to the severity of the disease and height of the leucocytosis.

Myelocytes were found by Turk in nearly all cases. They were most abundant about the time of defervescence, and once reached a proportion of 11.9 per cent. among 8800 cells.

Peculiar cells ("reizungsformen") of large or small size, with single or double nuclei, staining densely and with a brownish tinge by the triacid mixture, are described by Turk as of frequent occurrence, and as resulting from abnormal stimulation of blood-forming tissues.

Applications in Diagnosis and Prognosis. The very numerous situations in which the examination of the blood is of great value in the diagnosis of pneumonia have been pointed out by numerous writers. It is especially in such acute uncomplicated infections that the results of examination of the blood are to be relied upon, but even here a word of warning is needed against too implicit confidence in this or any other isolated clinical sign, *while it is especially unwise to transfer to other fields in blood analysis the rather positive rules which may usually be applied to the changes in the leucocytes in pneumonia.*

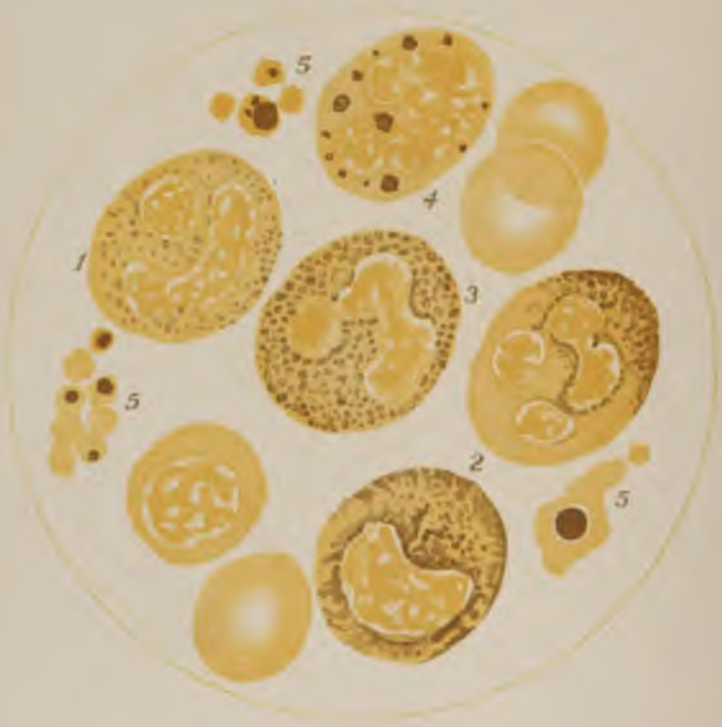
Barring mild cases, and those fatal ones in which there is no increase of white cells, leucocytosis is an invariable accompaniment of pneumonia, and its absence is, therefore, very strong negative evidence against pneumonia.

When present, leucocytosis excludes, with somewhat less certainty, a considerable class of diseases which are not usually accompanied by leucocytosis. These are principally typhoid and typhus fevers, malaria, acute tuberculous pneumonia, uncomplicated influenza, and the catarrhal pneumonia of influenza (Rieder).

In *prognosis* a slight leucocytosis indicates a mild case, while in severe cases a low proportion of leucocytes is an extremely unfavorable prognostic sign. This fact was first pointed out by Kikodse and v. Jaksch, although such cases had previously been reported by Halla and Hayem. Yet absence of leucocytosis appears not to be so unfavorable as was at first supposed. Out of fifty-seven severe cases without leucocytosis, reported by Halla, Hayem,[2] Laehr, Ewing, Sadler, Bieganski, Zappert, Turk, Billings, Cabot, only forty-four died, although all of them were unusually severe cases. Lambert points out that some severe cases may show absence of leucocytosis in the first examination, but later a well-marked increase, whereas a continuously low or decreasing number of white cells, as reported in three of his patients, is probably seen only in fatal cases.

The persistence of leucocytosis may be of value in distinguishing pseudocrises, but Bieganski, Turk, and others have seen the leucocytes fall during a pseudocrisis. Continuous or increasing leucocy-

PLATE XII.



Glycogenic Degeneration of Leucocytes.
(Iodine and Potassium Iodide in Mucilage of Acacia.)

Fig. 1. Polynuclear leucocyte showing early stage of iodine reaction.

Fig. 2. Mononuclear and polynuclear cells with circumscribed areas containing glycogen.

Fig. 3. Polynuclear leucocyte in advanced stage of glycogenic degeneration. From a case of purulent peritonitis.

Fig. 4. Large isolated granules of glycogen in a polynuclear leucocyte.

Fig. 5. Forms of extracellular glycogen.

tosis after defervescence often indicates a relapse or complication. The reappearance of eosinophile cells indicates that the lesion has passed its acme.

Bacteriology of the Blood in Pneumonia. That infection with the pneumococcus may often take the form of a general blood infection is indicated by the clinical course of virulent infections in man and animals and by the frequent occurrence of the micro-organism in the blood of the cadaver. Similar indications are found in the report by Netter and Levy of finding the pneumococcus in the blood of a dead fetus whose mother had died of septicemia, and by the discovery of the same germ in the milk of a nursing woman who was attacked by pneumonia (Bozzolo).

Numerous bacteriological studies of the blood during life have shown also that in a moderate proportion of severe cases the pneumococcus may be obtained in culture by the usual procedure.

Sittmann obtained the largest proportion of positive results, 4 out of 16 cases examined, three of which were fatal and gave signs during life of general septicemia. Similar cases of pneumococcus septicemia are reported by Belfanti, Netter, Marchiafava and Bignami, Goldscheider, Grawitz, Bacciochi, and others, and it has become evident that in this group of infections the pneumococcus is usually to be found in the blood during life. Other successful results were obtained *only a few hours before death* from pneumonia, and the presence of the pneumococcus in the blood in these cases must be regarded as an *ante-mortem* invasion (Boulay).

As regards the bacteriological condition of the blood in ordinary uncomplicated lobar pneumonia evidence has steadily increased which indicates that this disease is often a form of bacteremia, but the question cannot yet be regarded as settled. Most of the early observers were unable to isolate the pneumococcus from the circulating blood in any considerable proportion of cases. The following table represents the more important work in this field:

BACTERIOLOGICAL STUDIES OF BLOOD IN PNEUMONIA.

Author.	Year.	No. of cases.	Positive results.		Negative results.	
			Total.	Fatal.	Total.	Fatal.
Sittmann	1894	16	4	3	12	3
Kraus, Kuhnau	1897	21	2	2	19	
Kohn	1897	32	9	7	23	
James, Tuttle	1898	12	0	0	12	10
Sello	1898	48	12	10	27	9
White	1899	19	16	16	3	0
Beco	1899	49	7	5	42	15
Silvestrini, Sertoli . . .	1900	16	15	...	1	
Prochaska	1900	50	50	12	0	
Pieraccini	1900	28	11	..	17	
Landi, Cionini	1900	27	25	...	2	
Cole	1902	30	9	9	21	4

Prochaska's results were remarkable, as he claims to have found the pneumococcus in all of a series of fifty cases, twelve fatal. He sowed 10 c.c. of blood in distinctly alkaline broth. Sometimes, especially in cases with metastatic inflammations, *staphylococcus aureus* or *streptococcus* were found associated with the pneumococcus. Some of his examinations occurred as early as the second day. He naturally claims that bacteremia is invariable in the disease, but he found no relation between the leucocytosis and the grade of bacteremia and no relation to prognosis.

It appears premature to draw positive conclusions from these varying results. Fraenkel claims to have verified Prochaska's conclusions, but Cole, who sowed more blood than Prochaska, secured rather few positive cultures. None of the recent observers mention the difficulty of distinguishing the pneumococcus from the streptococcus.

From these studies it may be concluded that when pneumonia leads to metastatic inflammations the pneumococcus is frequently found in the blood; that in some cases the blood is invaded just before death; and that in uncomplicated pneumonia the pneumococcus is rarely found in the blood during the progress of the lesion, and that when it is present the disease is usually fatal.

DIPHTHERIA.

Red Cells. *The concentration of the blood* demonstrated to a slight degree in pneumonia is even more distinct in the highly febrile stages of diphtheria. Grawitz refers the tendency to a lymphogogic action of diphtheria toxin which he has observed clinically and experimentally to cause an increase in the specific gravity of the blood. Although Bouchut found an average of 4,300,000 cells in ninety-three cases, a distinct reduction, yet many of his observations were made late in the disease, and individual cases often showed slight polycythemia. Cuffer reported extreme polycythemia, 7,200,000 to 7,800,000 in three cases. Later observers, especially Morse and Billings, have closely followed the changes in the red cells, finding high normal proportions, 5,100,000 to 5,600,000 during the first week. In the second week Morse found even higher numbers, once 6,800,000, while Billings, although finding an average loss of 500,000 cells, encountered some cases with distinct polycythemia. With the fall in temperature slight anemia appears in many cases, but not in all. Bouchut and Billings observed losses of 2,000,000 cells in several cases.

Morphological changes in the red cells are not marked, but when anemia develops there is slight polychromasia and the usual deficiency of Hb. Nucleated red cells were noted by Engel. The Hb is slightly diminished in the average case, but may be considerably reduced in cases which have lost many red cells. Billings found an average loss of 10 per cent. in patients not receiving antitoxin.

The Leucocytes. The early observers, Bouchut, Cuffer, Pee, Halla, Limbeck, and Rieder, fully established the presence of leucocytosis in diphtheria, but only Bouchut attempted to deduce any rules governing its occurrence. He observed an increase in all cases, usually proportionate to the severity of the disease, but in septicemic cases the increase was less marked and the prognosis fatal. In view of the recent observations of many myelocytes or an extreme proportion of lymphocytes in diphtheria, Bouchut's statement that the blood of diphtheria may be "leukemic" is not far from the truth. More detailed studies were contributed later by Gabritschewsky, Morse, Ewing,[2] Billings,[2] Engel, Schlesinger, and Filé.

The leucocytosis of diphtheria resembles in most respects that of pneumonia. It begins very early when the disease develops suddenly, and reaches its highest point within one or two days, or begins more slowly and increases steadily for several days or until death. It probably begins earlier and reaches its acme sooner in more refractory individuals, with whom the prognosis is comparatively good.

The writer observed two cases *without leucocytosis* until the fourth and sixth days. In one of these the condition was probably referable to prolonged toxic hypoleucocytosis, and the patient died later. Not infrequently the white cells begin to rise only after defervescence, while long persistent leucocytosis is also of rather common occurrence. In favorable cases the leucocytes usually diminish steadily after the first few days. In *fatal cases* the leucocytes may steadily increase or may steadily decrease during the days before the fatal issue, or there may be no leucocytosis.

The height of the leucocytosis is usually considerable, 25,000 to 30,000 cells being frequently present in severe cases. The majority of fatal cases at some period show between 25,000 to 50,000 cells, hence the conclusion of Gabritschewsky that a high and progressive leucocytosis is a bad prognostic sign. Mild attacks, especially in adults, may not show any increase. The writer found 72,000 cells in one case with lymphocytosis, while in a peculiar case marked by hemorrhagic eruption Felsenthal counted 148,000—possibly an agonal hyperleucocytosis. The grade of leucocytosis is little affected by the fever, but in many cases is proportional to the extent and depth of the membrane. It may be distinctly affected by pneumonia, but the writer failed to find any uniform lymphocytosis when the cervical nodes were greatly enlarged, nor did the character of the infection, whether pure or mixed, appear to influence the leucocytes (cf. Filé). In general, the leucocytosis of diphtheria is less marked than that of pneumonia with equal temperature, and less than that of non-diphtheritic angina of equal extent (Filé).

Types of Leucocytes in Diphtheria. With well-marked leucocytosis the polynuclear neutrophile cells are usually much increased in numbers and proportions, but they very seldom reach the very high ratios seen in pneumonia, probably on account of the greater tendency of children to lymphocytosis. *Lymphocytes*, probably for the same reason, are relatively more abundant. A striking lymphocytosis occurred at the height of the disease in two of the writer's cases, in one of which the lymphocytes, large and small, formed 60 per cent. of 72,000 leucocytes, and in another 62 per cent. of 22,500 cells. In neither was there marked enlargement of cervical lymph nodes. Of quite different significance is the relative lymphocytosis without increase in total numbers which may be seen in the earlier stages of the disease, and was noted by Billings in a fatal case without leucocytosis, and by Rieder and Filé during convalescence. *Eosinophile* cells are usually reduced in proportions, often persist in low normal ratio, and frequently cannot be found at all. They are relatively more numerous than in pneumonia.

Engel made the interesting observation that *myelocytes*, both neutrophile and eosinophile, are frequently present in the blood of diphtheria, and that when these cells form over 2 per cent. the prognosis is very unfavorable. In cases which recovered the myelocytes never formed over 1.5 per cent., while in seven fatal cases they ran from 3.6 per cent. to 14.6 per cent. It should be added that the numbers of myelocytes varied greatly from day to day, and that eight of thirty-two patients died with few or no myelocytes in the blood.

Degenerative changes in the leucocytes in diphtheria are often well marked. The "leucocyte shadows" of Klein are seen in all severe infections and may become very numerous in fatal septic cases. Even before the cells become fragmented many of them, principally the polynuclear forms, show deficiency of chromatin and of neutrophile granules. The writer, examining the blood in 0.6 per cent. salt solution tinged with gentian violet, found a striking deficiency of chromatin in a case in which the leucocytosis disappeared before death. In the other septic and fatal cases also it was plainly apparent, although not demonstrable in less severe infections. This change in the leucocytes, which may be seen in other infectious diseases, and especially in acute leukemia, must be classed with the general cellular degenerations which mark the *status infectiosus*. Its significance in diphtheria has been fully discussed by Gabritschewsky, while Filé believes that these pale-staining cells are necrotic. The writer believes that, with careful technique, variations in the staining capacity of leucocytes may be made of value in prognosis.

An increased acidophile tendency of the neutrophile granules appeared to be demonstrable in some of the writer's fatal cases. Kanthack has also noted a similar change in the staining tendency of the pseudoeosinophile granules in rabbits which had received small injections of microbic poisons.

Effects of Antitoxin upon the Blood in Diphtheria. The red cells show no distinct or uniform effects from the injections of antitoxin, although in some healthy subjects there is a moderate reduction lasting a few hours. On the other hand, the use of antitoxin, by limiting the progress of the infection, tends to prevent further destruction of blood cells.

Within half an hour after the injection of antitoxin, the leucocytes, especially the polynuclear forms, if previously abundant, show a marked diminution, and in most cases, although the leucocytosis returns after twenty-four to forty-eight hours, it seldom reaches its previous grade.

The writer noted the reduction of leucocytes after antitoxin in all but two fatal cases, while Schlesinger found it in all of his examinations. It has also been noted by Fikl. In the writer's cases the loss was from 4000 to 15,000, but Schlesinger observed a loss of 20,000 within seven hours after the injection. In some fatal cases the use of antitoxin is followed immediately by distinct hyperleucocytosis (the writer, Gundobin). The most favorable cases show a steady diminution of white cells after the injection, but in many fatal cases this reduction is interrupted after the third or fourth day. In critical cases with many pale-staining leucocytes the writer found an improvement in the staining quality of many polynuclear cells shortly after the injection of antitoxin. In some fatal cases this change could not be detected.

CHAPTER XII.

THE EXANTHEMATA.

VARIOLA.

Red Cells. During the febrile period Hayem found very little change in the red cells, while in the stage of suppuration polycythemia was sometimes to be noted. Immediately after defervescence, when the destruction of blood cells through the septic process was no longer masked by the concentrating influence of fever, the red cells showed a sudden reduction. In a case of confluent smallpox there was a loss of 2,000,000 cells. In a fatal case before death there were 4,600,000 cells, while in another, markedly hemorrhagic, there were only 2,000,000.

Pick, from his study of forty-two cases, found very little change in the red cells throughout the entire course of the disease. He failed to find any severe anemia, although many of his cases were hemorrhagic and confluent. In severe cases which recovered the red cells remained normal before the eruption, almost invariably increased during the suppurative stage, even in hemorrhagic cases, and after defervescence or before death were rarely found much below their original figure.

The usual condition of the blood after smallpox appears to be, therefore, a mild form of chlorotic anemia, while under some rather unusual conditions this anemia may become severe.

Leucocytes. Although leucocytosis was demonstrated in the eruptive stage of variola by Brouardel, Hayem, Halla, and Pee, the detailed knowledge of the subject has been contributed principally in the studies of Pick, and of Courmont and Montagard.

Mild cases of *varioloid* with scanty eruption ran their course, under Pick's observation, with normal or subnormal numbers of leucocytes, but when suppuration occurred the leucocytes were increased, reaching in one case 20,000.

In nearly all of Pick's cases of variola there was distinct leucocytosis beginning with the appearance of vesicles, increasing as the exudate became purulent, and reaching its height when suppuration became most active.

In cases which recovered the leucocytes returned slowly to the normal as suppuration subsided. Abscesses frequently interrupted recovery and caused marked exacerbations of the leucocytosis. In seven of eight cases dying of pneumonia there was a rapid and steady decrease of leucocytes, although suppuration continued. The grade of leucocytosis was usually proportionate to the severity of the septic process.

Failing to find leucocytosis in the high febrile periods of the early stages of severe cases, Pick concluded that variola uncomplicated does not tend to produce leucocytosis, but that the usual leucocytosis is referable to secondary infection. This conclusion must be doubted.

Courmont and Montagard have also contributed an extensive study of the blood of variola, and their results require a modification in some respects of the conclusions of Pick. They observed a mixed leucocytosis with excess of lymphocytes in nearly all cases, beginning in the early stages of the eruption, reaching its acme in the pustular stage, and in uncomplicated cases slowly declining. In uncomplicated cases the leucocytosis was usually slight, and varied between 10,000 to 20,000, but once reached 33,000 before the development of any pustules. In confluent and fatal cases there were usually more than 20,000 leucocytes. In hemorrhagic cases the leucocytosis was usually more marked, and in two cases reached 39,000 and 41,000 on the fourth day, the day of death. In some fatal cases the leucocytes tended to diminish markedly toward the end of the disease, from 19,700 on the ninth day to 2500 four hours before death. In subjects who had never been vaccinated the leucocytosis was distinctly greater than in vaccinated subjects.

The increase affected principally the lymphocytes, the polynuclear cells, with few exceptions, being below normal in percentage, though usually increased in actual numbers.

They properly distinguish between pustulation and true secondary suppurative complications, such as boils and abscesses. The former condition did not increase the percentage of polynuclear cells, although the pustules constantly yielded streptococci in cultures. Boils and abscesses, however, usually ran the percentage of polynuclear cells above normal. They conclude from these observations that pustulation is not the result of secondary infection of the skin, but is a part of the true variolous process.

In a detailed study of the varieties of leucocytes occurring in variola, it appears that the majority of new cells are medium-sized lymphocytes which constitute usually 35 to 45 per cent. of the white cells. The authors attribute great importance to the presence of 5 to 10 per cent. of very large mononuclear non-granular leucocytes in the blood of variola. Neutrophile myelocytes were never absent, occurring in an average of 3 per cent. of all cells, with extremes of 2 to 16 per cent. Marked eosinophilia was observed in one hemorrhagic case which recovered under serum therapy. On the second day in a fatal case 15,000 white cells were counted, of which 3 per cent. were mast-cells. A few nucleated red cells were always to be found, except in infants, most numerously in hemorrhagic cases. As the diagnostic features of the blood in variola, much reliance was placed upon the presence of lymphocytosis with excess of very large cells and associated with neutrophile myelocytes in moderate proportion.

Very similar results were obtained by Weil, who found slight leucocytosis, 6000 to 10,000, in six cases; 6000 to 15,000 in thirteen cases; 15,000 to 20,000 in nine; 20,000 to 25,000 in three;

25,000 to 30,000 in three; 30,000 to 35,000 in one; above 35,000 in one. The leucocytosis was most marked at the period of vesiculation, while with pustulation the leucocytes increased or diminished or remained stationary. In cases which were fatal without complications there was usually a diminution of leucocytes before death. The increase of large mononuclear cells and myelocytes was characteristic, especially in cases ending in streptococcus septicemia. In the pustules he found 10 to 20 per cent. of mononuclear cells and myelocytes, and 30 to 60 per cent. of polynuclear cells. While claiming that the pustulation is not due to secondary infection he found that the pustules always contain streptococci, which make their appearance very suddenly, as on one day he would find none and on the next enormous numbers.

The writer examined the blood in twelve cases of variola at various stages. Distinct leucocytosis was found in only six of the cases, but the low proportion of polynuclear cells was always observed if complications were absent, and once at the height of pustulation only 21 per cent. of these cells were present. A few myelocytes, however, were seen in only five of the cases. It was found, as did Courmont and Montagard, that the low proportion of polynuclear cells exists in the blood when the cutaneous lesions contain pus and streptococci.

Until the contagium of variola is discovered and until the relation of the streptococcus to the disease is determined it will probably remain undecided whether the leucocytosis of the disease is referable to the true virus or to the streptococcus. It is certain that leucocytosis is absent in the very early stages in many cases of ordinary severity, but it is also true that the streptococcus is often multiplying in the cutaneous lesions before any pronounced leucocytosis appears in the blood, and when the peculiar increase of mononuclear cells is prominent.

In the marrow of variola Roger, Josué, and Weil found very little reaction in adult cases, corresponding to slight leucocytosis observed clinically. In children with bronchopneumonia leucocytosis was marked and the hyperplasia of the marrow was distinct. In the adult marrow they found an excess of mononuclear cells and diminution of polynuclears.

Biological Examinations of the Blood in Variola. In 1887 G. Pfeiffer called attention to the presence in vaccine lymph of small unicellular ameboid bodies, and the following year he found these same bodies in human lymph. These observations were soon confirmed by Rieck, and later by Ogata, who classed the bodies as *protozoa*, order *gregarinidæ*.

Guarnieri, in 1892, believed that he had cultivated the same bodies in the epithelial cells of the rabbit's cornea, and although Ferroni and Massari claimed that Guarnieri's parasites were degenerating nuclear products, some but not all subsequent observers have succeeded in verifying Guarnieri's conclusions.

In 1894 Pfeiffer reported the discovery of amebæ in the blood of smallpox patients, describing them as free in the plasma, about one-fourth the size of red cells, possessing one or more nuclei, and projecting pseudopodia.

Weber and Doehle have described in the blood of measles, scarlet fever, and smallpox, several forms of ameboid bodies which they believe to be developmental stages of a parasitic protozoön.

In 1897 Reed found pigmented ameboid bodies in the blood of vaccinated monkeys and children, and in the blood of variola, but he found the same bodies in normal subjects, and he could not demonstrate any nucleus.

Kruse, reviewing the evidence in 1897, ascribed great significance to Guarnieri's bodies in the epithelial cells of the cornea, believing that they represent the first stage in the solution of the etiology of variola, but he did not grant equal importance to the bodies found in the blood.

Widal and Bezancon are reported to have found streptococci in the blood of six cases of variola, and Arnaud in two primary hemorrhagic cases. The writer[3] sowed in broth 10 c.c. of blood in five cases of variola of ordinary severity and found all the cultures sterile. Only one of the cases died.

Serum therapy in variola has been attempted by Courmont and Montagard by the use of calf serum drawn fifteen days after vaccination. The treatment seemed to lessen complications, while the injections usually caused a diminution of the leucocytes in cases showing leucocytosis.

VACCINIA.

Billings, in fourteen infants, noted a moderate but distinct polynuclear leucocytosis, usually about 15,000, maximum 20,000.

Sobotka examined the blood of forty-three children after vaccination, making the following observations:

The red cells and Hb showed no constant variations, and usually remained normal.

Vaccination always caused a leucocytosis, beginning usually on the third or fourth day after inoculation and gradually falling until the seventh or eighth day, when the leucocytes were frequently subnormal. From the tenth or twelfth day a *secondary leucocytosis* regularly appeared, lasting two to six days, and showing close relation to the activity of the virus and the number of the pustules.

The height of the first leucocytosis varied between 12,000 and 23,000; that of the second between 10,000 and 17,500, while in the intermediate period the cells fell as low as 3500. In uncomplicated cases the lowest figures usually corresponded to the highest temperature, the leucocytosis preceding by several days the local and general manifestations of vaccinia.

Similar changes are noted in infants by Enriquez and Sicard, but they failed to detect the intermediate decline observed by Sobotka. In these cases the mononuclear cells numbered 60 to 70 per cent., the polynuclear 30 to 40 per cent., and once there were 3 per cent. of myelocytes. Of twelve cases in adults they found leucocytosis, 10,000 to 12,000, in only four. In seven rabbits vaccination, which proved fatal in two to three weeks, caused well-marked large-celled lymphocytosis.

VARICELLA.

Engel[2] found 67 per cent. of polynuclear cells, with absence of eosinophiles, in a child at the height of the pustular stage. Three days later, when most of the pustules were healed, the polynuclear cells had fallen to 47 per cent., and the eosinophiles risen to 16 per cent. The total numbers of leucocytes were not stated, but the above proportions indicate a moderate leucocytosis.

Nobecourt and Merklen, in fifteen cases, found no change in the red cells and little in the leucocytes. In seven cases there was an excess of large mononuclear cells, in six slight polynuclear leucocytosis, maximum 4500. The eosinophiles were nearly always diminished. In five cases there were 1 to 12.5 per cent. of myelocytes. Weil and Descos, from a study of twenty cases, concluded that varicella sometimes yields a slight polynuclear leucocytosis, maximum 13,500, while in thirteen of their cases the leucocytes were within normal limits. They state that the absence of large mononuclear cells and myelocytes will serve to distinguish varicella from variola. Stengel and White, in four cases, found distinct leucocytosis but once, and in a patient suffering from bronchitis.

From these somewhat discrepant results it will be seen that the blood in varicella may not differ in any particular from that of mild cases of variola.

SCARLET FEVER.

Hayem gave the first systematic account of the blood changes in scarlatina, finding a loss of about 1,000,000 red cells after defervescence, and a moderate leucocytosis in average cases, much increased by severe angina or by suppurative processes. Somewhat isolated observations were reported by many of the earlier blood analysts, Halla, Pee, Pick, Reinert, Sadler, while considerable series of cases were studied by Rieder and Kotschetkoff.

The *whole blood* suffers in a slight degree the usual effects of fever. The formation of fibrin is usually increased, especially when there are extensive angina or suppurative complications (Hayem). Heubner noted hemoglobinemia in one case, representing the septicemic type of the disease.

Red Cells. The gradual loss of red cells noted by Hayem was fully verified by Kotschetkoff, who found a reduction to 3,000,000 or lower in nearly all cases. The regeneration of the blood was slow and complete only after six weeks or longer. On the other hand, Zappert found less than 4,000,000 cells in only one of six cases, and very slight anemia was noted in a considerable number of cases examined at varying periods by Leichenstern, Arnheim, Pee, Pick, Reiner, Sadler, Felsenthal, and Vandenberg. When complications supervene, however, as nephritis or endocarditis, very severe anemia may rapidly develop. In such cases Vandenberg reports as low as 25 per cent. of Hb, 2,000,000 red cells, and specific gravity 1031. In the late stages of fatal cases this severe anemia may be referred to the widespread occurrence of the streptococcus.

Leucocytes. Kotschetkoff classified the leucocytoses in three groups, the *mild cases*, showing between 10,000 and 20,000 white cells, the *moderately severe cases*, with 20,000 to 30,000 cells, and *very severe and usually fatal cases* with a leucocytosis of 30,000 to 40,000 cells, while in some *rapidly fatal cases* over 40,000 leucocytes were found. Yet Rieder's ten observations never gave more than 25,000 cells, and usually less than 20,000, although some of his cases were complicated with pneumonia and croupous pharyngitis, and were fatal. Felsenthal's six cases in children were of moderate severity and showed 18,000 to 30,000 cells.

The leucocytosis begins one to two days before the appearance of the rash, reaches its height with or shortly after the full development of the eruption, and while in some cases rapidly falling with the eruption, usually continues for four to five days longer, and very often persists for days or weeks after the temperature has become normal. Mackie, who found leucocytosis in twenty-five cases, places the period of greatest leucocytosis in mild cases at the fourth day after the appearance of the rash.

The grade of leucocytosis seems in general to correspond with the severity of the disease, especially of the angina, but not with the height of the temperature. Complications such as lymphadenitis, otitis, nephritis, usually have little effect on the leucocytes (Kotschetkoff), but Pee observed two cases in which the leucocytosis increased when the lymph nodes began to swell late in the disease. Pneumonia caused but slight increase in some of Rieder's cases. Mackie counted 95,300 cells in a case of septic angina, and noted a steady decrease of white cells in a fatal case.

Types of Leucocytes. The percentage of polynuclear cells is in all cases much increased, varying from 85 to 98 per cent., according to the intensity of the disease, reaching the highest point on the second day of the exanthem, and thereafter slowly declining. In fatal cases the proportion of polynuclear cells falls but slightly, or soon regains or passes the original figure.

The eosinophile cells may show characteristic variations. In all but very severe cases they are normal or subnormal at first, steadily increase after two to three days, reaching a maximum of 8 to 15 per cent. in the second or third weeks, and thereafter, declining slowly, reach the normal figure about the sixth week. In ten cases Reckzeh found that they began to increase on the first day, reached an average of about 6 per cent. on the seventh to eighth days, thereafter gradually diminishing until the fourteenth day. In fatal cases they may disappear early in the disease. The *lymphocytes* are at first diminished, but later rise to normal proportions.

The above rules deduced by Kotschetkoff, while probably representing the average case, are not always applicable. Thus Weiss found no eosinophiles in one case at the height of the exanthem. Rille observed marked eosinophilia in a fatal case; Bensaude observed as high as 20 per cent. of eosinophiles in one instance; Klein reports lymphocytosis during convalescence; and many writers (Rieder, Turk) have described a high persistent leucocytosis, especially in those cases followed by nephritis or other complications. Turk has called attention to the remarkable change which the leucocytes undergo about the fifth day of the disease, when the polynuclear cells rapidly diminish and eosinophiles and lymphocytes rapidly increase. This "secondary leucocytosis" he likens to the somewhat similar phenomenon seen in variola.

Bacteriology. Although the specific contagium of scarlet fever has not been demonstrated, practically all observers who have dealt with the cadavers of such cases have found a coccus widely distributed in the viscera and blood. Many have found in this coccus certain characters which they believe could distinguish their particular micro-organisms from *streptococcus pyogenes*, while others, dealing apparently with the same germ, have been content to class it with the polymorphous streptococcus. Very few of these observers have attempted to demonstrate the presence of the germ in the blood during life, nearly all reports being concerned with its occurrence in the blood after death. Charlton, however, claims to have isolated a streptococcus from the circulating blood of all those cases, in a series of twenty-five, in which the examination was made during the first five days of the disease.

Baginsky and Sommerfeld have collected about all the available evidence which indicates that the only micro-organism constantly present is the *streptococcus pyogenes*, and that this germ is the specific contagium.

Kurth, d'Espine and Marignac, and Class, Gradwohl, and Page, have endeavored to maintain an independent position for certain cocci which they succeeded in isolating from the throat, skin, and cadaver, occasionally from the circulating blood.

Raskin found *streptococcus pyogenes* in the circulating blood in two of sixty-four cases, while all other cultures were sterile. Negative results were obtained in two cases by Sittmann and in four cases by Kraus.

At present it is impossible to decide what is the relation between the different cocci described, what is their relation to the disease, or how frequently they are to be found in the circulating blood.

MEASLES.

The red cells have been found in the great majority of cases to suffer little or no change, but a loss of Hb is usually demonstrable after defervescence. Reckzeh mentions the occurrence, in very severe cases, of anemia with unusual variations in the size of the cells, with microcytes, macrocytes, and normoblasts.

In adults uncomplicated measles seldom causes leucocytosis, but is characterized rather by hypoleucocytosis, reaching in one of Rieder's cases 2700 cells. From 4000 to 6000 cells are commonly seen. This fact was first noted by Pee, and has been confirmed by Pick, Rieder, Rille, Felsenthal, and others.

Considerable variations in the behavior of the leucocytes in the first days of measles are apparent in the numerous reports collected by Reckzeh.

Normal or slightly reduced numbers of white cells are usually present at the onset of the disease. Combe finds that the decrease begins during the last two days of the period of invasion and becomes more marked during the stage of eruption. Sobotka and Cazal, however, observed hyperleucocytosis before the eruption appeared.

At the height of the exanthem they are usually at their lowest figure (Pee, Turk), and return to the normal within a few days or a week after defervescence. When the bronchitis is severe there may be a moderate leucocytosis, Hayem finding 10,000 to 14,000 cells in such cases occurring in children. Rieder observed slight leucocytosis in a case complicated by catarrhal pneumonia. Cabot observed 9000 cells in two cases, one hemorrhagic. The writer found no leucocytosis in three cases occurring in malarious subjects. The malarial parasites reappeared, with chills, during convalescence.

The proportions of the various leucocytes show no distinctly abnormal changes. Turk found a rather high percentage of polynuclear cells during the fever, with diminution of small lymphocytes, and Reckzeh's tables also show a high proportion of polynuclear cells, 62 to 88 per cent., on the third and fourth days. Pee, Klein, Turk, and Combe noted an excess of large mononuclear cells appearing with the eruption. The eosinophiles are usually in low normal proportions during the early febrile period, and tend to diminish as the eruption declines. Yet Turk found nearly 5 per cent. during the second week of the disease.

Bacteriological examination of the blood was negative in ten cases examined by Barbier. Weber claims to have found in the blood of measles a protozoon, which he has also seen in variola.

Applications in Diagnosis. Typical cases of measles and scarlet fever may sometimes be distinguished from each other in their early stages by the examination of the blood. Yet, as Turk says, *the blood in measles strongly resembles that of a mild scarlatina*, as both show a nearly normal number of leucocytes and normal proportions of eosinophiles. Yet equally severe constitutional disturbance should give, on the second to third days, *leucocytosis, if scarlatinal; normal or diminished leucocytes, if from measles.*

In **German measles** there was no leucocytosis in two cases mentioned by Cabot.

CHAPTER XIII.

TYPHOID FEVER.

The whole blood commonly suffers concentration in the early stages of the disease as a result of the febrile process, while at any period profuse diarrhea and repeated hemorrhages considerably reduce the total bulk. This concentration is so marked and constant that the deep-red appearance of the blood drop in typhoid fever is a very characteristic differential sign between this disease and pernicious malaria.

Fibrin formation is usually deficient and may not be demonstrable at all, but with inflammatory complications this element may reappear and become abundant (Hayem, Turk).

Red Cells. During the febrile period the red cells usually show a slight and gradual decline. Yet the initial concentration of the blood often yields moderate polycythemia during the first two weeks or longer, so that the slight anemia is not to be noticed until the fever and diarrhea subside (Sadler, Felsenthal). Many observers have found over 5,000,000 red cells in individual cases at varying periods of the fever, while Thayer reports nearly 7,000,000 in the first, second, and third weeks. At such times a considerable grade of pre-existing anemia may be obscured by the febrile concentration of the blood. Even when distinct polycythemia does not result the same factors tend to keep up the proportion of red cells, so that in the vast majority of cases of typhoid fever the red cells number more than 4,000,000, and in order to demonstrate an anemia referable to the disease it is necessary to follow the case from the first. That the typhoid toxemia tends nevertheless to produce anemia is clearly shown in the large series of cases reported by Thayer and Da Costa, in which there is each week a progressive loss of red cells and Hb, the weekly loss averaging for the first five weeks 100,000 to 500,000 red cells and 5 to 6 per cent. of Hb.

The Hb suffers to a great extent, and in spite of the concentration of the blood most febrile cases register between 60 and 80 per cent. of Hb. Severe diarrhea or large hemorrhages may at any time cause marked oscillations in the red cells and Hb. After and often before defervescence the blood may show distinct anemia, which, with rapid defervescence, may appear suddenly (Arnheim, Zaslein, Boeckman, Laache). During convalescence the anemia slowly disappears, the Hb being restored much later than the red cells.

The *grade of anemia* observed after typhoid fever varies greatly with the character of the disease. Uncomplicated cases may show no distinct reduction of cells and very slight loss of Hb. In a considerable proportion of severe cases the red cells fall below 4,000,000 and the Hb below 70 per cent. Kohler, in twenty-nine cases, found an average loss of 19.4 per cent. of Hb in men and 20.4 per cent. in women, while the average of red cells for men was 4,030,000 and for women 3,580,000. Thayer's average for the fourth week was 4,200,000 and Da Costa's 3,500,000. Hayem, Thayer, Henry, and others have reported severe grades of pernicious anemia as sequelæ of typhoid fever. In one of Henry's cases there were only 804,000 red cells.

Morphological changes are usually not marked, but in severe cases Turk found megalocytes, small microcytes, and polychromasia in comparative abundance. Nucleated red cells may appear after hemorrhage.

Leucocytes. Numbers. In the first week uncomplicated cases nearly always show a normal number of leucocytes, but severe angina, bronchitis, enteritis, etc., may yield a moderate polynuclear leucocytosis. Klein and Aporti regarded this initial leucocytosis as of frequent occurrence, but most other observers have failed to encounter it. The writer has seen so many examples of moderate polynuclear leucocytosis in the first week of typhoid fever that he would warn the observer that the behavior of the leucocytes at this time is quite variable, and that complications can seldom be discovered to account for the leucocytosis.

In the second week the leucocytes usually show a distinct reduction, especially of the polynuclear forms, but the number of leucocytes found at this time is by no means constant.

In some cases the lowest figures of the disease are reached in the second week (Turk, Chetagurow), while in others there is no distinct reduction at this time (v. Jaksch,[2] Sadler).

In the third and fourth weeks the leucocytes usually continue to diminish until the acme of the disease is reached (Turk), after which they slowly increase. In many cases the lowest figures are reached at this period (Rieder, Turk, Thayer), but sometimes the reduction continues until after defervescence (Klein).

The average course of leucocytosis is shown in Thayer's collection of 826 examinations:

	Examinations.	Leucocytes.
First week	119	6442
Second "	258	6251
Third "	200	5528
Fourth "	117	5431
Fifth "	70	5510
Sixth "	25	5690
Seventh "	14	6132
Eighth "	14	6614
Ninth "	7	5057
Tenth "	2	5000

A relation between the leucocytes and the fever seldom exists, nor does the size of the spleen appear to influence the number or type of the leucocytes (Turk). That the reduction of the white cells is dependent on the action of the typhoid toxin is indicated by the further losses which commonly go with unfavorable turns in the disease (Rieder, Jez, Turk, Nagaeli). *It is therefore a general rule that the more severe the typhoid intoxication the lower is the count of leucocytes.* The reduction is seldom below 2000 cells, but 1000 to 2000 were reported by Hayem, Limbeck and Rieder, Cabot, and Kohler. The majority of cases at some periods show between 4000 and 6000 cells, and many fall below 4000.

Leucocytosis during the later course of typhoid fever is by no means uncommon. Da Costa claims that it is present at some period in about 10 per cent. of all cases. Usually a severe diarrhea from extensive ulcers, or hemorrhage, or pneumonia, or other exudative or suppurative complications, may be found to explain the increase. Yet several reported cases with leucocytosis have shown no such complications (Aporti), and a short experience at the bedside seldom fails to bring to light some moderate leucocytoses without apparent cause. Courmont and Barbaroux observed polynuclear leucocytosis (89 per cent.) throughout an uncomplicated fatal relapse, and in several cases at some time during the continuous pyrexia, usually at its acme, there was an increase of polynuclear cells. In grave cases they frequently found a series of elevations of the polynuclear cells, and conclude that the typical course of the leucocytes can be expected only in cases of moderate severity. On the other hand, leucocytosis may fail in the presence of pneumonia or other markedly exudative complications (Turk, the writer, Cabot). Kohler found 6200 and 4200 leucocytes in cases complicated by severe *bronchitis.* After *hemorrhage* in one case the white cells remained at 2600; in two others there was an increase of about 4000 cells. In eleven cases of hemorrhage Thayer observed a reduction of red cells, and after twenty-four hours a leucocytosis, maximum 24,800, in only half the cases.

Complicating *pneumonia* once reduced the leucocytes from 2600 to 1000; at another time they remained at 6300, and twice 11,200 and 11,800 cells were counted (Kohler).

Regarding the effects of the numerous other inflammatory complications of the disease it appears that the occurrence or failure of leucocytosis is quite as uncertain as after pneumonia.

Cold baths have long been known to cause temporary massing of leucocytes in peripheral capillaries (Winternitz). In twenty cases Thayer[2] found an average increase of 5346 cells, and a maximum increase of 17,000. With the return of peripheral circulation the excess of cells disappears.

Perforation usually causes polynuclear leucocytosis, but there may be no effect upon the leucocytes, or the percentage of polynuclear cells may rise without increase in their total number.

One may refer the failure of leucocytosis after perforation to the low condition of the patient or to the discharge into the peritoneum of much septic material, or to violent infection by the streptococcus, as in three cases reported by Thayer. As Russell found, leucocytosis in these cases may be due not so much to the perforation as to the subsequent peritonitis, and may, therefore, be delayed until after other signs have become urgent. The leucocytosis when present may be very evanescent, and its presence may in general be regarded as more favorable than its absence.

Types of Leucocytes. During the first week of the disease the persistence of a normal proportion of lymphocytes without noticeable increase of neutrophile cells is a very characteristic feature of typhoid blood. Barring the occasional occurrence of initial polynuclear leucocytosis, from the end of the first week there is a progressive increase of the lymphocytes and diminution of the neutrophile cells. At first the lymphocytes do not pass beyond high normal limits, but during the third, fourth, and fifth weeks, or later, they usually occur in distinct excess. While mononuclear cells seldom fall below 25 per cent. at any stage of the disease, a proportion of 40 to 60 per cent. is often seen. Among these cells the proportion of small lymphocytes is often striking, but usually the medium-sized leucocytes are most abundant. In some cases there is a very marked absolute lymphocytosis. In one of the writer's cases at Montauk the blood resembled that of lymphatic leukemia, a resemblance rendered still more striking by the enormous size of the mesenteric nodes at autopsy. With the increase of mononuclear cells the numbers of polynuclear cells gradually fall, reaching 60 to 65 per cent. in most cases and occasionally a much lower figure (35 per cent., Jez; 20 per cent., Klein). The excess of lymphocytes usually persists during the first weeks of convalescence, and may not reach its acme until this time, while Ouskow found that normal relations were not restored until the tenth or eleventh week. Nagaeli describes the development during convalescence, especially in children, of a well-marked lymphocytosis with a moderate increase of neutrophile cells. In children this lymphocytosis was most marked two or three months after the fever, while in adults it was less marked and disappeared by the end of the second month.

Eosinophile cells are usually absent or very scarce during the febrile period, but reappear shortly before (Nagaeli), during, or after defervescence. Aporti found as high as 18 per cent. during the intermittent pyrexia at the end of the disease, but they are usually much less abundant.

Degenerative changes in the leucocytes occur as usual, Jez reporting large numbers of pale leucocyte shadows in severe cases. Glycogen was found in the leucocytes in increased quantity between the twelfth and twentieth days by Livierato.

Applications in Diagnosis. The morphological examination of the blood is often of great assistance in the diagnosis of typhoid fever and the diseases which simulate it. The writer knows of no clearer illustrations of this fact than those which he reported[1] in 1893, and which have been paralleled from numerous later observations and by many writers both before and since.

Suppurative processes, if active, may usually, but not always, be distinguished from typhoid fever by the presence of polynuclear leucocytosis. Yet it should be remembered that slow suppuration, or the mere presence of pus the secretion of which has ceased, are frequently unaccompanied by leucocytosis or are even marked by relative lymphocytosis. Thus the writer found 50 per cent. of lymphocytes among 11,000 cells in a case of large abscess of the liver with mild typhoidal symptoms, and 45 per cent. of lymphocytes among 7000 cells with the chest full of pus. With increasing experience, therefore, the writer believes that most careful observers will recognize the partial justice of Grawitz's claim that in difficult cases the blood examination here often fails to be of service.

Among the conditions which, by the almost invariable presence of leucocytosis, may be distinguished from typhoid fever, are bacterial endocarditis, suppurative appendicitis, and pneumonia. Malaria of sufficient gravity to simulate typhoid fever can nearly always be distinguished by the anemic appearance of the blood drop as compared with the deep red, concentrated blood of typhoid fever. An astonishing change in the blood was often noted at Montauk when typhoid fever developed in subjects of malarial cachexia, and in no instance were malarial parasites found in such concentrated blood. In acute paroxysms the discovery of the parasite is usually possible, but chronic malaria without parasites in the blood may simulate typhoid fever.

Between miliary tuberculosis, typhoid fever, and some forms of meningitis, Widal's test is required and the enumeration of leucocytes is of little value.

From an extensive study of the leucocytes in typhoid fever Nagaeli draws the following conclusions regarding *prognosis*. The prognosis is *favorable:* (1) When eosinophiles are present at the height of the disease, or reappear in the second or third stages of the febrile period. (2) When lymphocytes begin to increase after the severest toxemia is past. (3) The diminution of neutrophile cells is slight only in more favorable cases. *Unfavorable signs* are: (1) Very small numbers of all varieties of leucocytes. (2) Failure of leucocytosis with complications.

Bacteriological. In the hope of developing a method of early diagnosis of typhoid fever many biological studies of the blood have been undertaken. *Aspiration of the spleen* has given successful results in the majority of cases (Chantemesse and Widal, Redtenbacher, Lucatello, Neisser, Bruschettini), but this dangerous procedure cannot be generally adopted. The results of Silvestrini, who found the typhoid bacillus in the aspirated blood of the spleen in four cases in which there were no characteristic intestinal lesions, are most suggestive.

In *blood drawn from the rose spots*, while early attempts were rather unsuccessful, Neuhaus and later observers have been able to isolate the typhoid bacillus in nearly all cases often before the appearance of Widal's reaction. Scholz and Krause recommend that the test be made as soon as possible after the spots appear, since they found that the bacillus disappeared after three to five days.

Richardson plants the material curetted from five or six incised spots, as well as the blood which oozes from the incisions. Freezing by ethyl chloride diminishes the pain of the operation.

Bacteriological studies of blood in typhoid fever usually gave negative results until Kuhnau reported eleven positive cultures in forty-one cases, examining blood drawn from the basilic vein at the acme of the disease.

In 1900 Auerbach and Unger pointed out that large dilutions in broth are necessary to annul the bactericidal action of the blood, and they secured positive results in seven of ten cases by planting ten to thirty drops in 300 c.c. of broth. The growth was at first often scanty and the bacilli non-motile and thread-like, but grew abundantly in subcultures. Later it was shown that the bacillus is very constantly present in the blood in early stages of the disease, but may disappear in the third week or with defervescence. Thus, Schotmuller was successful in forty of fifty cases; Castellani, in twelve of fourteen cases; Cole, in eleven of fifteen, and Hewlett, in twenty of twenty-four; while Courmont,[1] who was successful in nine consecutive cases, claims that the bacillus can always be isolated from the blood before the decline of the disease, although more than one culture may be required.

Not only have these recent studies demonstrated that typhoid fever is probably always a form of bacteremia, but it has been shown that the characters of the bacterial strains concerned in typhoid fever vary considerably, each producing highly specific agglutinins in the blood (Achard and Bensaude, Widal, Gwyn, Cushing, Schotmuller, Kurth, and Buxton).

That the disease may assume the character of a mixed septicemia is indicated by a considerable number of reports of the presence in the blood of other bacteria, especially *staphylococcus aureus* (Loison, Sittmann, Kraus, Pennato).

Serum Therapy in Typhoid Fever. For a history of this subject and a consideration of its problems and the various attempts that have been made to meet them, the reader is referred to the author's review,[4] which includes literature up to 1902.

The most noteworthy results in serum treatment of typhoid fever are those of Chantemesse. This investigator began in 1896 to inject horses with very toxic cultures of *bacillus typhosus*, secured and handled anaërobically. In 1901 he treated 100 cases with the serum thus obtained which had very high agglutinative properties and strong preventive powers in animals. The mortality was 6 per cent., the chief value of the treatment proving to be in the relief of symptoms and in shortening the course of the disease. Three of the fatal cases died of perforation, against which the treatment was of little avail. Two of the fatal cases were critically ill of pneumonia when treatment was begun. The serum caused rapid defervescence and improvement in all symptoms. Occasionally the temperature rose again after ten days of improvement, but the relapse, though often severe, was promptly controlled by the serum. The injections caused distinct leucocytosis for twenty-four to forty-eight hours, after which the neutrophile cells diminished and eosinophile cells reappeared.

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CHAPTER XIV.

WIDAL'S TEST.

THE history of the development of Widal's test from the discovery of the phenomenon of Pfeiffer in 1894 through its first application to the diagnosis of typhoid fever by Pfeiffer and Kolle, and Gruber and Durham, in 1896, up to its more practical modification by Widal, has now been very fully reviewed in numerous monographs and treatises on bacteriology and need not here be repeated. It may only be said that while the scientific basis of the test had been firmly laid by previous studies, up to the time of Widal's communication there had been no indication that the specific reaction could be obtained from small quantities of serum and at the beginning of the disease, while the use of dried blood and the introduction of the test into municipal laboratories, thus rendered possible, was first accomplished by Johnston, of Montreal.

Description of the Reaction. When to a few cubic centimetres of blood serum of a patient suffering from typhoid fever is added a few drops of an actively motile culture of *bacillus typhosus*, the bacteria are precipitated in flakes, leaving the supernatant fluid clear. When the sediment is examined microscopically it is found that the bacilli have lost their motility and are agglutinated in masses, while with very intense reactions they may be broken up into granules or even completely dissolved. When the test is performed with a drop of dissolved blood inoculated by means of a platinum loop with a small portion of broth culture, all stages of the reaction may be followed under the microscope.

With very intense reactions the agglutination is found to be complete as soon as a cover-glass can be adjusted over the drop and the specimen placed under focus. The bacilli are motionless, they are nearly all gathered in characteristic clumps, which stretch in a network irregularly through the field, and very soon, in some cases, evidences of subdivision or solution of the bacteria may be detected.

If the reaction is less complete, the clumps are less compact and a few motile bacilli may be found between the masses. With high dilutions of the blood, all stages of the reaction may be followed. The first change noted is a partial loss of motility. The bacilli cling to one another after struggling to free themselves, but gradually entangling their neighbors. In the course of ten or fifteen minutes the process results in the gradual clumping of all bacilli, or, according to the strength of the reaction, a variable number of actively motile bacilli may remain between the loose masses.

When the reaction is present in traces only, much longer time is required for the appearance of small clumps, but these reactions must be discarded as unreliable. All experienced observers have insisted that distinct islands of clumped bacilli must be present to constitute a positive reaction, *and Fischer and others maintain that all bacilli must be rendered quiescent before the result can be regarded as positive.*

Wilson describes as a partial reaction one in which well-defined clumps appear in every field, but composed of motile bacilli, while the edges of the hanging drop are not free from bacilli. He found such reactions in thirteen different diseases.

Pseudoreactions. While partial reactions occur under many conditions when the technique has been faultless, certain effects simulating the true agglutination may appear in specimens containing not a trace of the specific principle on which the test depends. In *old cultures* many loose clumps of bacteria may be transferred by the loop from the culture tube and be mistaken for an effect produced by the serum. *Normal blood* or *serum*, in somewhat concentrated form, exerts a marked influence upon the motile typhoid bacillus, causing it to lose its motility after a time, but failing to develop typical clumps. *If the specimen has been allowed to dry* many bacteria adhere to slide or cover-glass or gather at the thickening edges of the specimen, and sometimes these collect in motionless groups. Such clumps always lack the reticular arrangement seen in the true reactions. A considerable variety of confusing appearances will meet the beginner, resulting from the *formation of fibrin* and *the incomplete solution of masses of red cells.*

Technique: Methods of Obtaining the Body Fluid. Although specific reactions have been obtained from many secretions, excretions, and other fluids of the body, only the blood, its serum expressed from the clot, or the fluid gathering in blisters, yield sufficiently constant results to be available in clinical diagnosis.

THE WHOLE BLOOD. When the examiner can reach the bedside there is no better method than to employ the whole blood diluted in known proportion by distilled water. In order to obtain an accurate dilution a medicine dropper may be graduated in one and ten or twenty drop marks, as suggested by Cabot, but such dilution cannot be very accurate. The writer has long used for this purpose the leucocyte pipette of the Thoma-Zeiss hematocytometer, which gives an exact dilution of 1 : 20 or 1 : 50, or more, and which is itself a very convenient instrument for handling the blood. Levy and many others have devised special tubes adapted to this purpose. The chief objection to this method is the failure of the blood to completely dissolve in dilution of 1 : 20 or 1 : 50 of water, but no other method can furnish more exact dilution, which is essential in accurate work. This difficulty may be avoided by laying the pipette on the side until the corpuscles settle.

Blood dried on a glass slide and dissolved by mixing with water is now used in probably 90 per cent. of all examinations. One or two drops are touched to a clean glass slide and dried in the air. Such a specimen protected from moisture may be kept for days, transported for long distances, and being easily obtained, even by the patient himself, furnishes by far the most practical method of obtaining the necessary material for the test. Its great disadvantage is the impossibility of securing an exact dilution of the blood when it is redissolved. This difficulty, while theoretically insurmountable, proves to be of comparatively little moment in practice, since an experienced worker can learn to control the dilution by the color of the drop. Also, when imperfectly dissolved the detritus of red cells may obscure the field, although it should not fail to be distinguished from masses of bacilli

Some observers have found less powerful and constant effects from dried blood than from serum (Widal, Delepine, Johnston), but for diagnostic purposes Park finds the two methods of nearly equal value. Nevertheless, for accurate studies of variations in the reaction, the use of dried blood is inapplicable, although it will probably always remain in extensive employment for simple diagnostic purposes.

SERUM. 1. *The Bulbed Tube.* A capillary tube with central bulb is filled by capillary attraction from the expressed drop, the ends are fused, and when the blood coagulates a few drops of serum are exuded. A great many modifications suited to individual purposes have been suggested for this method. It offers considerable technical difficulties, but furnishes serum without delay.

2. *The Blister.* Serum may be obtained by means of a Spanish fly blister in from six to eighteen hours, and in quantity sufficient for extended examination without involving much pain or inconvenience. The fluid may be drawn in a sterile test tube and afterward divided in measured quantities, or it may be sealed in a capillary tube. For detailed study blister serum is superior to all other fluids. For measuring serum the "mixer" of the hematocytometer is well adapted, and the test may be performed in the hanging drop or in the test tube.

The Culture. Considerable difference in the response of different cultures of bacilli have been demonstrated by Kolle, Achard and Bensaude, Durham, v. de Velde, and Ziemke. Although Foerster found very little difference in a considerable series of cultures, most observers agree that attenuated cultures are more readily agglutinated. Müller succeeded in greatly diminishing the agglutinability of a given species and at the same time increasing its virulence by passing it through animals. It appears that for each culture there is a limit beyond which normal serum is positively inert, while at the same dilution typhoid serum exerts a specific action (Foerster). While any pure culture may be used, Müller and others have shown that a freshly isolated culture is not suitable for diagnostic use, and must first be transplanted many times in artificial media. The best results have been obtained in New York City by a so-called "Pfeiffer specimen" imported by Park. A stock culture of this particular growth is kept in sealed tubes of nutrient agar in the ice chest and replanted every few months. From this growth the specimen employed in testing is obtained in broth culture grown for twenty-four hours at a temperature of 37° C. In such broth cultures the bacilli are completely isolated and actively motile, so that the slightest change in motility or tendency to clump can be detected by comparison with a control specimen.

Dead Bacilli. Widal first observed that cultures of *typhosus* which had been killed by heat (57° to 60° C., thirty to forty-five minutes) had lost little or none of their capacity to agglutinate, and this fact has been abundantly attested by others. Many have therefore preferred to use a dead culture killed by heat, or by 10 per cent. formalin, or by thymol, or by corrosive sublimate, 1000 to 5000 (Park), thereby dispensing with the trouble of preparing a fresh culture. Such an expedient, however, can hardly meet with general adoption, on account of the difficulty of keeping the dead bacilli well isolated, and because the gradual loss of motility is a desirable and reassuring feature of the test in mild reactions, which are just the ones in which uncertain clinical signs render a positive test more desirable.

Attenuated cultures have been recommended by Johnston, who found that in using dried blood pseudoreactions were not infrequent with active virulent cultures, while broth cultures planted from old agar stock were less susceptible and did not respond to healthy or non-typhoid sera.

Paratyphoid Strains. From the observations of Achard and Bensaude, Widal, Gwyn, Cushing, Schotmuller, Kurth, Coleman and Buxton, and others, it has been shown that the bacteria in typhoid fever may show certain characters, such as the fermentation of sugars with the production of gas and acid, which gives them an intermediate position between *typhosus* and *bacillus coli communis*. Buxton has shown that these intermediate forms, isolated by himself and obtained from the above observers, may be still further classified into three rather distinct groups. It appears, also, that the agglutinins developed by these strains are highly specific, that of one group usually having little effect on other groups. Buxton found a certain grade of interaction between the members of his groups. These considerations suggest that where the blood is negative to *typhosus* it should be tested with various paratyphoid strains. The writer attempted to do this in a short series of cases of suspected typhoid fever, but where the reaction was negative to *typhosus* it was negative also to the paratyphoid cultures.

Degree of Dilution of Serum and Time Required. Although it has been believed that the serum reaction in typhoid fever depends on the presence in the blood of a peculiar substance which exerts a specific action on the typhoid bacillus, yet it has been found that in a considerable proportion of cases healthy serum, or that of patients suffering from other diseases, contains substances which exert a very similar influence upon typhoid cultures. *In typhoid fever, however, the serum may usually be diluted twenty to fifty times, or often very much further (1000 to 2000, Widal[2]) without destroying the reaction, while in other diseases the reactions usually disappear with dilutions of 1 : 10, or almost certainly with dilutions of 1 : 20.*

The *element of time* is also of great importance in distinguishing the specific typhoid reaction from that produced by other sera.

While a distinct reaction often occurs instantly with typhoid serum, blood of other diseases almost never acts immediately, but requires one-half to one hour or longer to disclose its effects.

Consequently, in order to demonstrate the specific typhoid reaction, it is necessary to increase the dilution and to limit the time.

"The results obtained in the New York City Health Department laboratories and elsewhere have shown that in a certain proportion of cases of typhoid there occurs a delayed moderate reaction in a 1 : 10 dilution of serum or blood; but very rarely, if ever, excepting in typhoid fever, does a complete reaction with this dilution occur within five minutes. When dried blood is used the slight tendency of non-typhoid blood in 1 : 10 dilution to produce agglutination is increased by the presence of fibrinous clumps and perhaps by other substances derived from disintegrating red cells. From many cases examined by Fraenkel, Stein, Foerster, Scholz, ourselves, and others, it has been found that *in dilution of 1 : 20 or more, a decided quick reaction is never produced by any febrile disease other than typhoid infection, in which it often occurs in dilution of 1 : 50*" (Park).

Yet even this dilution appears insufficient to eliminate all sources of error, and there has been a constant tendency among experienced workers to increase the grade of dilution. Grunbaum placed it at 1 : 30; Simon, at 1 : 30 or more; Stern, after a large experience, at 1 : 40; and Mewius, at 1 : 60.

From these authoritative opinions it becomes necessary to prescribe a dilution of at least 1 : 20, better 1 : 30, while the limit of time should not be greatly extended. Even these limits will be found insufficient to avoid all possible error, and in doubtful cases one should use a dilution of 1 : 40 or 1 : 60, increase the time to one or two hours, and require a distinct result.

Occurrence of the Reaction. Statistics vary considerably regarding the dates of occurrence and the constancy of the reaction in typhoid fever.

Cabot collected over 3000 cases of supposed typhoid fever, of which 95 per cent. gave the reaction at some period of their course, while in 2500 control cases, not typhoid, 2 per cent. gave positive reactions.

Park reports positive results in a large series:

During the first week, in	20 per cent. of the cases.
" second "	60 " " "
" third "	80 " " "
" fourth "	90 " " "
" second month, in	75 " " "

In 88 per cent. of hospital cases in which repeated examinations could be made, a definite reaction was obtained at some period of the disease.

Wilson reports further on the work of the New York City Department of Health, as follows: In 256 examinations the test was positive in 8 cases not diagnosed as typhoid, while 3 were malaria, and 1 puerperal sepsis with tuberculous intestinal ulcers. Of 1115 cases 117 which gave no reaction proved to be typhoid.

The earliest date of appearance of a positive reaction has not been and cannot well be determined in the human subject, but in animals inoculated with dead typhoid bacilli the reaction appears between the third and eighth days. In man a positive reaction has been found by Johnston and Taggart, and by Fraenkel, on the second day, but the reactions were not sharp. Levy found the first distinct reaction on the sixth day in the human subject inoculated with dead typhoid bacilli. It may be found before the appearance of rose spots, splenic tumor, or the diazo reaction in the urine. During the course of the disease the reaction may continue without interruption, or may be absent one day while present the next. It may be absent entirely in mild cases, may appear only in a relapse (Lichtheim, Breuer, Thoinet), may disappear entirely after a few days (Elsberg), or may persist for months or even a year. Incomplete reactions have been reported after many years (Stewart). It not infrequently appears for the first time during convalescence or may exhibit a sudden increase at this time. According to Widal, in the majority of cases the reaction disappears by the fifteenth to thirtieth day of convalescence, but Stewart believes that it persists at least one year in 50 per cent. of the cases, for two years in 25 per cent., and for ten years in 5 per cent. His conclusions can only apply to very indistinct reactions.

In the blood of the fetus delivered during the course of typhoid fever, several observers have failed to find any agglutinative power (Mahnt, Stahelin), but Schumacher[2] claims that agglutinins are always present in the fetus during the last month of pregnancy, but that their persistence is of short duration.

Relation of the Reaction to Other Features of the Disease. In some cases the reaction is most intense at the height of the disease, as in cases observed by Jemma. Widal and Sicard, who found that in the active stage a dilution of 1 : 60 or 1 : 80 does not usually prevent the reaction, noted a marked weakening as convalescence proceeded, some cases failing to react with dilution of 1 : 10. They noted a reaction in one case with a dilution of 1000 or 12,000. Foerster, who found a reaction in dilutions varying from 1 : 60, 1000, or 5000, could not detect any relation between the intensity of the reaction and the severity of the disease. Pfeiffer and Kolle, and Foerster have shown that the agglutinating power has no relation to the bactericidal activity of the serum.

Courmont[2] has contributed an elaborate study of the course of the agglutinative power of the blood in typhoid fever, reaching some important conclusions regarding prognosis and offering evidence that the agglutinative power of the blood is closely connected with the defensive reaction of the organism. His conclusions are as follows:

In the great majority of cases of typhoid fever of ordinary or moderate severity the power of agglutination reaches a maximum at the period of defervescence, or during the first days of apyrexia, and falls, often rapidly, with the fever, or during convalescence. Agglutination curves that are continuously low (less than 1 : 200) are rarely seen in cases of moderate severity, but frequently in very toxic forms of the disease which are prolonged or fatal. Very irregular agglutination curves with premature fall are rare in moderate cases, very frequent in toxic and fatal cases. In some prolonged cases the curve of agglutination rises rapidly shortly before defervescence. In relapsing cases the agglutination curve varies, but usually follows the same types as in single attacks. It is usually higher in the relapse than in the initial attack if the patient recovers and lower if he dies. In mild and irregular types of the disease the power of agglutination is very variable and usually low or evanescent.

Effect of Typhoid Serum upon the Colon Bacillus. Although Fraenkel in a large series of cases reported that typhoid serum has no marked power to agglutinate the colon bacillus, and recommended the use of this serum in the separation of colon from typhoid bacilli, this claim has not been verified. Stern and Biberstein found, very shortly, five cases of typhoid fever of which the blood serum agglutinated the colon bacillus even more powerfully than the typhoid, and many other observers, including Park, Rodet, Courmont, Ustvedt, Bensaude, and Kuhnau, have had similar experiences in many cases. Baumgarten, therefore, concludes that this method can no longer be accepted as yielding positive proof of the identity of *bacillus coli communis*. It nevertheless remains true that a bacillus which on cultural characters falls between the typhoid and colon groups, is almost certainly *coli communis*, if it is not agglutinated by a well-tested and active typhoid serum. The possibility that infection by the colon bacillus is intermingled with that of *bacillus typhosus* in enteric fever has been abundantly considered by several writers. The safest method of identifying the colon bacillus is not by the serum of a case of typhoid fever, but by the serum of an animal which has been inoculated with a pure culture of *bacillus typhosus*.

Reactions in Conditions Other than Typhoid Fever. In view of the fact that positive reactions have been obtained both with healthy serum and in diseases other than typhoid fever when a dilution of 1 : 10 was used, many observers have recommended that higher dilutions only be employed. Thus Schultz obtained complete reactions in eleven of 100 cases of various febrile diseases with a dilution of 1 : 10; in seven cases with a dilution of 1 : 15, and in three with 1 : 20, while a faint response followed a dilution of 1 : 25 in a single instance. The time limit, however, was one to two hours. In single cases complete reactions with dilutions of 1 : 10 or more have now been reported in so many cases that it is no longer possible to refer the results to faulty technique.

Some of these conditions are as follows: septicemia (Ferrand); malaria (Bloch, Villiers, Catrin, and the writer); pneumonia (Kasel and Mann); tuberculosis (Wesbrook, Jez, d'Espine); otitis (Stern); influenza (Wesbrook); typhus (Park); meningitis (v. Oordt); normal serum (Kuhnau).

In many of these cases the dilutions were from 1 : 10 to 1 : 30, or more, and the clumping was prompt and complete.

In several cases of chlorosis Kohler claims to have secured a reaction in a dilution of 1 : 40, and in a case of severe anemia at 1 : 50.

A large percentage of cases of jaundice have been found to show high agglutinative power over *bacillus typhosus*, which Greenbaum refers to the presence of taurocholic acid.

In Weil's disease Eckhart obtained positive reactions in two cases, and Zupnik, in four of six cases of Weil's disease, two of cholelithiasis, one of cholangitis, and one of carcinoma of the liver, all the subjects being jaundiced.

Stewart, using dry blood in 538 cases, found typhoid lesions at autopsy in five which failed at three periods during the disease to give a positive reaction. Thompson also reports six negative results in 163 cases, while a slight reaction was obtained in 12 per cent. of various conditions other than typhoid fever. Ustvedt encountered a peculiar epidemic of typhoid fever among soldiers in which four of fifteen cases failed to yield a reaction. Total failure of reaction, under full precautions, is also reported in single cases by Artaud, Haushalter, Schumacher, Fischer, and others.

The significance of these negative results have been made apparent by the work of various observers, who have shown that a considerable number of bacterial strains, each developing highly specific agglutinins, may give rise to the symptoms of typhoid fever.

Value in Diagnosis. The difficulty in determining the value of Widal's test in diagnosis arises principally from the divergent opinions regarding the essential features of a complete reaction. Although the belief in the specific quality of Widal's reaction has been abandoned, the test remains an extremely valuable diagnostic procedure under several conditions.

It must be granted at once that absolute certainty cannot be ascribed to any test unless the dilution has been very high, certainly not less than 1 : 60, with a time limit of not more than fifteen to thirty minutes.

A positive result with much less dilution (1 : 30) must stand as *almost certain evidence* that the disease is typhoid fever. Such results are usually obtainable, however, only after the disease is well established and its clinical symptoms distinct. Accordingly the chief value of the test lies in its capacity to distinguish, during the height of the illness, certain conditions, such as acute gastritis, tuberculosis, meningitis, and pneumonia, which may simulate typhoid fever.

The application of the test in the *early diagnosis* of typhoid fever has, in the experience of most observers, proved disappointing. It is rarely to be found until other signs render the diagnosis very probable, when the blood test may often be added, as any other isolated clinical symptom, to the evidence for or against typhoid fever.

The writer believes, however, that the combination of an indistinct serum reaction, diminution of fibrin, absence of leucocytosis, and presence of relative or absolute lymphocytosis, can very rarely be demonstrated in the early stages of any obscure febrile disease except typhoid fever. The morphological examination of the blood is, therefore, a valuable adjunct and control in the application of Widal's test, and the writer believes that in doubtful cases it should never be omitted.

Negative results are of very moderate import until the third or fourth week, and unless often repeated, while some reported cases, often fatal, have failed at any time to yield the reaction. In case of a negative result in a case strongly resembling typhoid fever, one should if possible test the blood with various paratyphoid strains.

Summary. The most reliable *method* for clinical purposes is the use of fresh blood diluted with water in a graduated pipette, and inoculated as a hanging drop preparation. Blood or blister serum should be employed for more extended study, while the use of dried blood, sometimes necessary, gives very slightly less reliable results.

The *culture* should be grown in broth at 37° C., twenty-four hours old, actively motile, and of moderate virulence. A recognized brand should be obtained if possible, and the behavior of all newly isolated cultures should be tested before use, and replanted until they become readily agglutinable.

A *positive reaction* consists in the complete immobilization of all bacilli and the compact clumping of the great majority.

A *time limit* of five minutes is all that is necessary, with dilutions of 1 : 20, in the great majority of positive reactions. With higher dilution (1 : 40) the time may be extended to thirty minutes, and with dilution of 1 : 60, to two hours (Mewius).

A dilution of at least 1 : 20 is required in all positive reactions, with which, however, occasional errors will occur. Dilutions of 1 : 30 or 1 : 60 are to be strongly recommended, for the higher the dilution the more certain are all positive results.

A negative result is of little import unless obtained during the height of the disease (third to fifth weeks) and on repeated examinations. In the great majority of cases the reaction disappears within a few weeks after convalescence, and several authentic cases, usually fatal, have not given a positive reaction at any time.

The agglutinative power of typhoid serum is usually slight and transient in mild cases, marked and persistent in cases of average severity, low or variable in toxic cases, but bears no constant relation to the gravity of the disease, and gives no certain prognostic indications.

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CHAPTER XV.

MISCELLANEOUS INFECTIOUS DISEASES.

SEPTICEMIA, PYEMIA, OSTEOMYELITIS.

Red Cells. In no other disease do the red cells suffer destruction so constantly and to such an extent as in the toxemia of diffuse inflammation caused by the common pyogenic bacteria. This fact was very early noted, Braidwood finding shrinkage of the red cells and absence of rouleaux, Mannassein remarking on their reduced diameter, and Quincke and Patrigeon observing extreme loss of Hb in cases of pyemia.

Hayem and Toenissen placed the average loss of red cells in ordinary septic fever at 200,000 to 1,000,000 per week, while a continuous diminution was found to persist as long as suppuration continued. That the loss of red cells is often very much more rapid than Hayem supposed is shown by Grawitz's remarkable case, in which fatal puerperal sepsis combined with considerable hemorrhage reduced the red cells in about twenty-four hours to 300,000.

Various forms of acute septicemia not infrequently reduce the red cells below 2,000,000, but none appear to act more violently than does puerperal or uterine sepsis. Hayem[2] found only 1,450,000 cells, 20 per cent. Hb, in a recent case of puerperal sepsis. Cabot reports 1,800,000 cells from a "suppurating fibroid." The writer found 1,600,000 cells and 20 per cent. Hb in a septic form of endometritis, not puerperal.

When the suppurative process is localized the red cells are usually found to be only slightly affected, but with the first appearance of the "septic" condition the reduction of red cells promptly begins, Roscher finding evidences of diminished concentration of the blood within the first few hours. In empyema, suppuration in wounds, pelvic abscesses, appendicitis, peritonitis, etc., the red cells are seldom markedly reduced, while the usual effects of fever are present and polycythemia is often found. The Hb is, as usual, more susceptible than the red cells, and its loss is usually out of proportion to that of the red cells, the Hb-index being invariably low.

Timofjewsky found that nucleated red cells appear promptly in the blood of dogs after moderate injections of pyogenic bacteria, in one instance reaching the enormous proportion of 25,698 per c.mm. Turk found considerable numbers of nucleated red cells in two cases of septicemia from cystitis. In cases of intense puerperal sepsis the writer has usually been able to find a few nucleated red cells, but they have never been very numerous.

In *chronic suppuration* the red cells tend to diminish as long as the discharge continues. In a case of chronic empyema of one year's duration the writer found 1,800,000 cells and 25 per cent. of Hb, but an old pelvic abscess discharging a small quantity of pus for two years had induced very slight anemia.

Morphological changes in the red cells in septicemia are usually present in moderate degree. Most cases show a pure type of secondary chlorotic anemia with marked loss of Hb. The reduced diameter of the anemic cells, previously noted, was distinct in the writer's case of septic endometritis, but did not approach the grade seen in some forms of primary disease of the blood. In severe cases many cells show granular degeneration, but polychromasia has, in the writer's experience, not been prominent. Marked differences in the size and shape of the cells do not appear, as a rule, until after the second or third week of a severe septic process.

Leucocytes. A considerable number of cases of asthenic septicemia run their course without leucocytosis, or with distinct reduction of white cells. Such cases have been reported by Limbeck, Krebs, Cabot, Turk, and, usually proving fatal, their significance is similar to cases of pneumonia, diphtheria, etc., with hypoleucocytosis. In Turk's case, and in one observed by the writer, while the leucocytes were subnormal, the proportion of polynuclear cells was very high.

Da Costa reports absence of leucocytosis in twelve of twenty-one cases of severe septic processes, and the writer has observed a prolonged puerperal case with severe chills in which leucocytosis failed to appear.

The great majority of cases, however, show pronounced leucocytosis, which is usually in proportion to the severity of the disease. Among the reported cases showing no leucocytosis are doubtless some in which the examination of the blood was made during the antemortem decline in the number of leucocytes.

Regarding the more minute factors determining the grade of leucocytosis little is definitely known, and the examination of the blood must be interpreted according to the circumstances in each case.

While the rule that suppuration induces leucocytosis is almost invariable, it must be remembered that leucocytosis may promptly disappear when the exudation ceases, and that suppurations involving mucous surfaces may induce very slight leucocytosis.

The polynuclear leucocytes are almost always in high proportion in cases of sepsis, but Klein has recently described a case of hemorrhagic septicemia[1] with 76 per cent. of eosinophiles in the pleural exudate and 40 per cent. in the blood.

Chemistry. The loss of Hb has already been mentioned as one of the chief alterations in the blood of septicemia. Its solution in the plasma and the occurrence of hemoglobinemia is observed in severe cases with rapid destruction of blood. In cases of puerperal sepsis, or pyemia in infants, the solution of Hb may reach a very extreme grade. In a puerperal case the writer found the viscera discolored, and so great was the deposit of pigment that the liver and spleen closely resembled, in gross and microscopic appearance, the organs of pernicious malaria. The increased globulicidal action of the serum in these cases, to which the solution of red cells must be referred, has been described by Maragliano and others.

An increased tendency toward crystallization of the Hb has been noted by Bond, who, in examining fresh specimens, found a rich deposit of such crystals about the edge of the cover-glass.

Roscher noted in severe cases a very rapid lowering of the specific gravity of the blood, beginning a few hours after the initial symptoms. In fatal cases the dry residue of the whole blood fell to 15 per cent., while in favorable cases the loss of solids was usually much less. This rapid and extreme loss of albumins Roscher and Grawitz regard as a valuable prognostic sign. The serum also usually showed a pronounced loss of albumin, proportionate to the severity of the septic process, its dry residue falling from 10.5 per cent. to a minimum of 6.25 per cent. Yet in a very severe case the dry residue of the serum was 13.1 per cent. of its weight, which Grawitz explains as resulting from the solution of Hb.

In various forms of pyogenic infection Livierato found an excess of *glycogen* in the blood, even when the temperature was normal. Goldberger and Weiss also describe in the leucocytes during abscess-formation a *reaction to iodine* either in the form of a diffuse stain or a granular deposit. They also find many brownish staining extra-cellular granules after fractures, and believe that the somewhat obscure "iodine reaction" of the blood may be made of value in the diagnosis of abscess, fractures, etc.

Bacteriology. The frequency with which specific bacteria can be isolated from the circulating blood in cases of septic infection, in spite of the vast amount of study devoted to the subject, still remains a matter of doubt.

There are, on the one hand, a large number of studies of the blood in pyemia, septicemia, puerperal fever, osteomyelitis, etc., reporting the discovery of bacteria in a large proportion of cases, while in many others, reported by competent observers, very uniformly negative results were obtained.

On reviewing many of these reports it appears that the majority of them must be set aside as unreliable.

Rosenbach, Garrod, Raskin, Brunner, Blum, Czerniewski, Eiselberg, Cantu, Roux and Lannois, Bommers, Stern and Hirschler, and others, all used blood squeezed from the finger, and when they attempted to demonstrate the same germ in the viscera after death, were not always successful. Many of their successful cultures were made only a few hours before death. When accurate details are given, as by Czerniewsky, it appears that contaminations by clearly non-pathogenic germs frequently occurred.

On the other hand, Brieger, drawing blood from a vein by a sterilized syringe, obtained negative results in five cases of puerperal sepsis. Sanger, by the same method, in three cases of osteomyelitis found *staphylococcus aureus* or *albus* in the blood, but cultures from an inflamed joint gave only *streptococcus pyogenes*. Likewise Kraus reports six positive results in ten cases of puerperal sepsis, but one was examined postmortem, and two showed only *staphylococcus albus*. Cannon, who secured a number of successful cultures from the blood in puerperal sepsis, osteomyelitis, wound infection, etc., took some of his specimens from a puncture of the skin, and Petruschky, who usually obtained some bacteria, drew the blood through the "disinfected" skin by means of a wet cup. Sittmann, accepting as genuine the results of previous investigators, and adding nine positive cases of his own, concludes that in septicopyemia the bacterial agent is always to be found in the blood, and that its presence does not insure a fatal issue.

Somewhat striking conclusions may be drawn from the reports of Bertelsman, who secured fifty-four negative and forty-seven positive results from examinations in 101 cases of various forms of suppuration. In twenty-seven cases the streptococcus was obtained, and in fifteen staphylococci. Important features of his study were the uniformly positive cultures in osteomyelitis (six cases), the demonstration of bacteremia in panaritium, and in five of nine cases of urethral fever, in phlegmonous erysipelas, and in three cases of cryptogenic streptococcus septicemia which recovered, while in several instances it was shown that bacteria were present in the circulating blood for short periods and in rapidly decreasing numbers. In the absence of much greater detail than accompanies the report, such results cannot be accepted without confirmation.

Old observers, as Kuhnau, do not accept the conclusions of Sittmann and do not admit much significance in the isolation from the blood of *staphylococcus albus* or the *colon bacillus*. Kuhnau examined the blood repeatedly in twenty-three cases of septicopyemia, nineteen of which were fatal. In two *streptococcus pyogenes* was obtained, in one *staphylococcus aureus*. Several cultures gave non-pathogenic germs, among them *staphylococcus albus*, which was obtained from the blood, but not from the abscesses after death. The only successful cultures were made shortly before death, or in one case during a chill.

White also found *streptococcus pyogenes* only three times and *staphylococcus aureus* once in eighteen fatal cases of sepsis.

The results of Kuhnau's study cannot fail to raise the suspicion that many investigators have been too lax in technique, or have not fully identified their species.

From the review of the above studies the writer draws the following conclusions:

1. The only reliable method of obtaining blood for bacteriological examination during life is to draw it from a vein, in quantity not less than 5 c.c., through the thoroughly sterilized, or, better, the incised skin, by means of a sterile syringe or aspirator.

2. In the great majority of cases of local or general septic infection, septicopyemia, septicemia, pyemia, diffuse suppuration, osteomyelitis, etc., bacteria are present in the circulating blood *only for short periods and at infrequent intervals, most frequently during chills.*

3. A few hours before death from septic infection, various bacteria, some of which may not be active in the original process, make their way into the circulation.

4. In a very moderate number of cases of septic infection, especially those which are not attended by local abscess formation, the bacterial agent may be isolated from the blood during the progress of the disease.

APPENDICITIS.

Red Cells. From the observations of Da Costa in 139 cases it appears that anemia of moderate grade exists in nearly all cases of appendicitis. It is least marked in catarrhal cases, in thirty-eight of which Da Costa found an average of 4,180,000 cells, with some exceptions showing much greater loss, while the Hb commonly registered 20 to 40 per cent. below normal. Anemia is most marked in cases with abscess of long standing, in which the toxemia frequently causes a loss of 25 to 45 per cent. or more of Hb and a reduction of the red cells below 4,000,000. In Da Costa's series a reduction to 2,000,000 red cells was twice observed, once with a catarrhal and once with a suppurative lesion, while more than 5,000,000 cells were found in twenty cases.

Leucocytes. The behavior of the leucocytes is extremely variable. While *catarrhal* or *simple exudative* appendicitis usually fails to show leucocytosis, Da Costa found over 15,000 cells in five of forty-five such cases, average 8987, and once 17,100. This leucocytosis he attributes to local peritonitis or to conditions apart from the appendicitis.

With *necrotic lesions, abscesses, gangrene,* and *general peritonitis* there is well-marked leucocytosis in nearly all cases, except in the fulminant and asthenic type of the disease, in which the leucocytosis may fail. The majority of all cases fall in this category, as did thirty-three of seventy-two reported by Cabot, and ninety-two of 139 by Da Costa. While most of these cases show more than 15,000 white cells, and many more than 20,000, a considerable number fall below 15,000 within the limit frequently seen in simple exudative processes.

As in pneumonia, etc., very marked or very slight leucocytosis may be found in grave cases calling urgently for operation, while the sudden transformation which follows perforation from a necrotic process of small extent commonly fails to give warning by a disturbance of the leucocytes. Cabot reports four cases of general peritonitis without leucocytosis and two with slight increase (14,800, 16,000).

In following the course of appendicitis the daily examination of the blood may bring to light some important considerations.

A steadily increasing leucocytosis usually indicates an extending local process.

After an abscess has been formed the leucocytes commonly fluctuate between distinctly high limits, rising abruptly if peritonitis develops. As the abscess becomes walled off the leucocytes slowly diminish, and when pus is evacuated a favorable course is indicated by a rapid decrease of leucocytes.

In chronic cases without active formation of pus the leucocytes are usually subnormal (Bloodgood).

It, therefore, appears that the interpretation to be placed upon the examination of the blood in appendicitis, especially in its early stages, is extremely variable. With leucocytosis ranging between 8000 and 16,000 cells, the condition may rather frequently be: (1) simple exudative appendicitis; (2) necrotic inflammation with perforation and abscess; (3) a large abscess; (4) general peritonitis.

The blood changes follow no definite rule in those dangerous forms of the disease which pursue a mild course for a time, but suddenly develop peritonitis from perforation. Nor can the question of operation in frank cases be decided from the examination of the blood alone, which indicates only what damage actually exists, while the operation is intended to meet the conditions which may later arise. One of Cabot's cases with 33,000 white cells recovered without interference. After the period of invasion is passed and the character of the lesion established, the indications of the blood have proved much more reliable.

In differential diagnosis a positive conclusion seems to be warranted only in those cases which show well-marked leucocytosis, from the presence of which it is possible to rule out nearly all forms of typhoid fever, most but not all cases of fecal impaction, and nearly all forms of abdominal neuralgia. Hubbard, Da Costa, and Cabot have offered some very practical comments on the limitations of blood analyses in this field.

ABSCESS FORMATION.

Active suppuration in a confined space is almost invariably accompanied by distinct leucocytosis. Cabot has remarked on the high leucocytosis usually observed with very limited suppurative foci in subcutaneous, submucous, or interstitial connective tissues, and reports very considerable increase of white cells, with furuncle, carbuncle, and abscess of vulva, vagina, middle ear, uterus, Fallopian tube, ovary, lung, brain, parotid gland, neck, and in pectoral, psoas, and perinephritic regions. The writer's experience in a considerable variety of such conditions fully accords with these reports, leucocytosis having been present in the active stages of all such processes, but subsiding slowly or rapidly after operation or after the exudative process had ceased. An important exception to the rule was found in abscess of the liver with mucopurulent exudate. (See Liver.)

Glycogenic degeneration of the leucocytes, according to Locke's observations, occurs in considerable degree with all large abscesses, but is not a reliable indication of the presence of pus, since it may be absent with small abscesses, and disappears in forty-eight hours after the abscess is drained.

ERYSIPELAS.

A slight diminution of red cells and Hb was observed in severe cases by Malassez, Hayem, and Reinert. In mild cases the fibrin is not affected, but in severe forms of the infection its formation is much increased (Turk).

Leucocytosis occurs in nearly all cases, but Rieder reported one instance of facial erysipelas with a temperature of 39° C. and 6800 white cells. Turk observed one case of considerable extent with temperature 38.5° to 40.2° and the leucocytes between 7000 and 8900. Later the temperature rose to 40.5° and the leucocytes to 10,100. Zappert, also, observed two febrile cases with subnormal numbers of white cells (5500 to 6500). Hayem found between 7000 to 8000 leucocytes in very mild cases, but 12,000 to 20,000 when the rash was extensive.

Most of the cases of Pee, Rieder, Ehrlich, Limbeck, and Cabot gave leucocytosis of moderate grade, but in proportion to the severity of the disease. Higher leucocytosis was observed by Halla in uncomplicated facial erysipelas, and in a case complicated by pneumonia (23,000). Suppuration raised the count to 39,600 in a case of Reinert, and to 59,400 in one of Epstein. Chantemesse and Rey found that the leucocytes and the temperature vary as a rule together, although in some cases high fever was unaccompanied by leucocytosis.

When the total increase is considerable the polynuclear cells are much increased in proportion, but with slight increase the polynuclear cells are usually unaffected. Eosinophiles are usually diminished or absent (Zappert, Turk). Scanty myelocytes may also be found during the active stages, 5 per cent. of these cells having been recorded by Turk.

Negative bacteriological examinations of the blood in erysipelas are reported by Petruschky in three cases and by Kraus in one case. Bertelsman had negative results in three severe cases, and positive cultures in two fatal cases of phlegmonous type.

ACUTE RHEUMATISM.

A much increased formation of fibrin has been noted by many observers, Halla, Hayem, Berggrun, Turk. Garrod failed to find any uric acid in the blood of acute rheumatism, and Salmon demonstrated the absence of lactic acid.

Red Cells. It is a general clinical rule that patients who pass through an attack of acute rheumatism become distinctly anemic. In many *cases of ordinary severity* the loss of red cells is slight, while the Hb falls to 80 or 65 per cent.

In *more severe cases* Hayem seldom found more than 4,000,000 cells. Sorensen found an average of 4,160,000 in eight cases. In *prolonged and relapsing cases* the red cells not infrequently fall slightly below 4,000,000. Yet in few of the reported studies are the examinations sufficiently extensive to show that such anemia is referable to the acute disease. Turk, while admitting the usual presence of anemia even during the febrile period, saw a distinct increase in red cells beginning with defervescence, and in Cabot's cases it does not appear either that there was any uniform loss of red cells or that the cases of long standing were invariably the more anemic. It seems, therefore, that the severity of post-rheumatic anemia has been overestimated.

The Hb suffers more severely and much more constantly than the red cells. Cabot's average in thirty-one cases was 67 per cent., and a few cases fell below 60 per cent., while Turk found between 60 and 80 per cent. Prompt use of salicylates prevented the loss of Hb in a case of Lichtenstern's, and apparently also in one of Cabot's. Da Costa reports two very anemic cases with 26 and 30 per cent. of Hb, and 1,240,000 and 1,600,000 red cells.

In convalescence the restoration of Hb remains considerably behind that of the red cells.

Leucocytes. In mild cases without exudation there is usually no distinct leucocytosis (Pee, Rieder). When fever and swelling of joints exist there is almost always an increase to 10,000 or 15,000 cells. Turk insists that when the leucocytes reach 20,000 or more there are nearly always complications, such as pleurisy, pericarditis, or pneumonia. Hayem also found 17,000 to 18,000 cells in moderately severe attacks, and as high as 25,000 only in extremely severe and cerebral types of the disease. Cabot reports 21,000 to 31,000 cells in six cases, one complicated by acute endocarditis, while in the others no complication was mentioned. The writer, from the examination of forty cases, in 1893, can support Turk's statement, having found signs of pneumonia, or pericarditis, or hyperpyrexia, whenever the leucocytes rose above 20,000. The pneumonic signs, however, were not always those of complete consolidation.

With defervescence the leucocytes promptly fall to normal, and in relapses are much less affected than by initial attacks (Turk).

Types of Leucocytes. When the white cells do not greatly exceed 10,000, the proportions of the various forms are not much disturbed. With distinct leucocytosis the proportion of polynuclear cells rises to a considerable height. Eosinophile cells are absent only in the early stages; later, in spite of fever and exudation, they are always present in moderate numbers, while after defervescence most cases show a distinct tendency toward eosinophilia (Turk, Achard, Loeffler). In one of Turk's cases there were 13.8 per cent. of eosinophiles, and in another 8.33 per cent. shortly after defervescence. This observer believes that a high proportion of eosinophile cells during the febrile period is a good prognostic sign, and occurs principally in self-limiting cases.

Bacteriology. In several studies, reviewed by Sittmann, various pathogenic bacteria were obtained from the circulating blood, but these cases have later been classed as examples of septicemia and not of articular rheumatism. The principal bacteriological studies of the blood of acute rheumatism are those of Sittmann, Singer, Kraus, and Kuhnau. Sittmann obtained negative results from repeated cultures in five cases, and Kraus in twelve cases. Singer conducted an elaborate study of the blood and urine in sixty cases, but while he found *staphylococcus albus* in several instances, this germ was probably a contamination from the skin. In one case *streptococcus pyogenes* was isolated, but the history shows the patient to have been suffering from hemorrhagic septicemia and endocarditis.

Even more conclusive were the totally negative results obtained by Kuhnau in sixty-seven cases representing all types of the disease and many complications.

In the etiology of rheumatism several theories are at present actively maintained. Singer urgently claims that the disease is a mitigated form of pyemia from infection by *streptococcus* or *staphylococcus pyogenes*. A considerable number of observers, including Leyden, Triboulet, Wasserman and Meyer, and Poynton and Paine, have isolated from the blood and tissues a diplococcus which reproduces the disease in rabbits more or less perfectly and which they regard as the etiological agent. Singer thinks that this diplococcus is the *streptococcus pyogenes*, but it decolorizes by Gram's method.

Achalme and various other French observers have endeavored to prove the importance of an anaërobic spore-bearing bacillus obtained in several cases from the heart's blood and tissues. Others regard this bacillus as a contamination from the media. Predetchensky, who found the streptococcus in the blood in two of five cases, failed to secure any trace of Achalme's bacillus in large quantities of blood with liquid media and complete anaërobiosis. It is generally believed that bacterial invasion is a secondary phenomenon, and that while the disease may be of bacterial origin the circulating blood is nearly always sterile. At present it is impossible to decide between the claims of Singer and the results of Kuhnau, and those of Poynton and Paine, Triboulet and Coyon, or Achalme.

TONSILLITIS.

Follicular tonsillitis usually causes moderate leucocytosis, seldom rising above 15,000 (Halla, Pick, Pee), yet in mild cases it may be absent (Cabot). In phlegmonous tonsillitis more marked leucocytosis, reaching 20,000 cells or more, usually occurs (Pee, Rieder).

The leucocytosis is generally more marked than in diphtheria with equal constitutional disturbances (Pee).

WHOOPING-COUGH.

Meunier has observed, in thirty cases of pertussis in children of various ages, a pronounced or extreme leucocytosis which "*far exceeds the increase found in any other afebrile disease of the respiratory passages.*"

The grade of leucocytosis varies with the age of the patient, being most marked in children under four years, and usually reaching a slightly lower figure in children from four to seven years. It appears in the catarrhal stage before the characteristic cough, when its demonstration may be made of diagnostic value, and disappears slowly with the improvement of the disease. It is little influenced by complications. The average leucocytosis was 27,800, but in several cases 40,000 cells were found, and in one 51,150. The lowest figure obtained during an active period was 15,500. The proportion of lymphocytes was always high, averaging 53.8 in a portion of the cases, while the polynuclear cells maintained a low average (39 per cent.), and the eosinophiles were scarce or normal in number. Meunier refers the lymphocytosis to hyperplasia of bronchial lymph nodes.

De Amicis and Pacchioni also report marked leucocytosis in whooping-cough, beginning in the first days of the disease, reaching its height in the spasmodic stage, and being sometimes prolonged after the cessation of the typical cough. Cabot found 12,600 white cells and 78 per cent. Hb in a girl aged six years. In four cases in children between the ages of two and six years Stengel and White noted leucocytosis—12,145 to 34,667—of which the polynuclear cells constituted 30 to 40 per cent.

INFLAMMATIONS OF SEROUS MEMBRANES.

Pleurisy, Pericarditis, Peritonitis.

Serous inflammations of the large serous membranes usually cause a slight increase of leucocytes in the blood during the acute febrile stages. Thus Hayem found between 7500 and 12,000 leucocytes in acute pleurisy; Rieder 11,000 to 13,000; and some of Cabot's early cases registered as high as 15,000. Higher leucocytosis belongs to the more severe inflammations, with temperatures reaching 101° to 104° F. In two cases of serofibrinous pleurisy Limbeck found, with a temperature of 38.8° C., 18,000 to 19,000 leucocytes, and with a temperature of 41.5° C., 22,000 cells. The grade of leucocytosis excited by fibrinous pleurisy, pericarditis, etc., has apparently not been determined. The inflammation stands in an intermediate position in point of intensity.

With purulent processes the leucocytosis is usually much higher. In 1893 the writer found no difference in the leucocytosis of pneumonia from that of idiopathic empyema and purulent pericarditis. All the cases of pericarditis were complicated by pneumonia or rheumatism and gave very high counts (maximum 60,000). The writer has seen one case of very rapidly fatal empyema, yielding *streptococcus pyogenes* in cultures, in which there was scanty purulent exudate and slight and diminishing leucocytosis.

In purulent peritonitis, also, leucocytosis is not infrequently absent.

After the exudative process has ceased the leucocytosis subsides, very rapidly in the case of serous exudates and usually more slowly with purulent processes. The majority of serous effusions are therefore encountered, as in Halla's and Cabot's cases, when the leucocytes are normal in number; nor is it rare to find extensive empyema with no excess of white cells in the blood.

Tuberculous inflammations of serous membranes, when pure and uncomplicated, are usually not accompanied by leucocytosis.

In serous pleurisy it is seldom possible to distinguish the quiescent stages of simple serous effusions from beginning tuberculous exudates, and the examination of the blood cannot prove of much value in diagnosis.

Further considerations of the changes in the blood in inflammations of serous membranes is to be found in the sections dealing with appendicitis, rheumatism, tuberculosis, etc.

GONORRHEA.

In acute gonorrheal urethritis the red cells remain unaffected. A moderate leucocytosis is usually observed in severe febrile cases, and is markedly increased by many complications, including cystitis, epididymitis, orchitis, etc.

The polynuclear cells are in the usual excess, while eosinophiles, although sometimes very abundant in the urethral discharge (Bettman), usually remain within moderate limits (0.5 to 11.5 per cent.) in the blood (Vorbach). Bettman, however, while finding that there is no relation between the eosinophile cells of the discharge and those in the blood, believes eosinophilia is usually present in gonorrhea, *especially in posterior urethritis.* He found 25 per cent. in the blood in a case of gonorrheal epididymitis.

In gonorrheal rheumatism the changes in the blood are similar to those of the idiopathic joint affections.

The gonococcus has several times been isolated from the blood in gonorrheal endocarditis (q. v.). Panichi, in two cases of gonorrheal rheumatism without endocarditis, reports the isolation of the gonococcus from blood sowed on human blood serum. He believes that the culture of the blood in order to be successful must be made at the time of invasion of the rheumatic symptoms.

YELLOW FEVER.

From Jones' interesting observations about yellow fever, it would appear that anemia is infrequent, that fibrin formation is deficient, that the globulicidal action of the serum is very greatly increased, and that cholemia is responsible for some of the changes observed in the red cells before and after shedding. "When a drop of blood from a yellow-fever patient falls upon blotting-paper, a dark, brownish ring due to diffusion of bile-stained plasma spreads about the central mass of blood cells" (Jones).

Pothier examined in some detail the blood of 154 cases. The red cells never fell below 4,280,000, and in one fatal case were normal. The Hb suffered a considerable loss during the course of the disease, registering between 50 and 72 per cent. In the above fatal cases 90 per cent. of Hb was found. The restoration of the Hb was slow.

Morphological changes in the red cells were usually absent, but the presence of normoblasts was noted in a few specimens.

The *leucocytes* fell between 1660 and 20,000. The polynuclear cells were found in high proportions in some of the slides from these cases examined by Cabot, while in others their proportions were normal. Eosinophiles were very scarce and myelocytes were in two cases. The writer examined dry specimens of the blood of two rapidly fatal cases of yellow fever brought from Rio Janeiro by Dr. J. M. Masury, and noted slight hemoglobinemia and hypoleucocytosis.

Pothier tested the *action of the serum* of some of his patients on cultures of Sanarelli's bacillus, but the results did not indicate the presence of any specific power in the blood.

Archinard and Woodson claim to have found the blood in yellow fever to agglutinate the bacillus of Saranelli in 75 per cent. of the cases. Reed and Carroll[1] have shown, however, that while this reaction occurs in a small proportion of cases (34 per cent.), it is in those same cases exhibited also with the bacillus of hog-cholera, with which they identify the bacillus of Sanarelli.

Through the labors of Reed, Carroll, Agramonte, and Lazear, the importance of Sanarelli's bacillus in yellow fever has been set aside, and it has been shown that the specific virus of the disease is present in the circulating blood and is transferred from one subject to another by means of the mosquito, *culex fasciatus.*

An interval of about twelve days or more after contamination appears to be necessary before the mosquito is capable of carrying the infection. They succeeded in producing yellow fever in a series of non-immune subjects through the agency of mosquitoes under conditions precluding the possibility of error. They also transferred the disease by injections of blood taken during the first and second days of illness.[3] The blood itself was found sterile. In three cases blood heated for ten minutes at 55° C. failed to cause any symptoms when injected in doses of 1.5 c.c. in non-immune subjects. Of three subjects receiving injections of 1.5 c.c. of yellow-fever serum passed through a Berkfeld filter two developed the disease. From one of these two cases serum was obtained and injected into a non-immune subject who also developed the disease. Reed and Carroll, therefore, conclude that the contagium of yellow fever is a very minute organism capable of passing through a Berkfeld filter.

TYPHUS FEVER.

In four cases the writer found between 5000 and 9000 leucocytes. The patients were adults, the examinations were made during the high fever of the early period of the disease, and at least two of the patients died.

Tumas followed one fatal case during a three weeks' course, observing a steady reduction of red cells from 4,400,000 to 3,100,000, of Hb from 80 to 50 per cent., and hypoleucocytosis 9600 to 1600.

EPIDEMIC INFLUENZA.

The scanty reports of the condition of the blood in epidemic influenza indicate that the disease in uncomplicated form fails to cause leucocytosis. This fact, while in accordance with the catarrhal character of the essential lesions, is somewhat difficult to reconcile with the acute infectious nature of the disease. Grippe appears to be the only bacterial disease, beginning acutely with marked chill, which fails to induce leucocytosis.

The absence of leucocytosis in grippe was first demonstrated by Rieder,[2] who, in seven cases, found that the white cells at the acme of the disease were reduced in number (7000 to 2800). In the catarrhal pneumonia which complicates the disease and which is distinguished by signs of incomplete consolidation, he found little or no leucocytosis (maximum, 13,000). In lobar pneumonia following grippe the usual leucocytosis was observed. In 1893 the writer found no difference in the leucocytosis of lobar pneumonia following grippe from that of primary pneumonia. Cabot reports the examination of the blood in sixty-seven cases, most of which showed normal or reduced numbers of white cells, and when there was leucocytosis (maximum, 14,000) complications were usually present.

Bacteriological Examination. In a considerable proportion of cases Canon, Klein,[2] Hirschfeldt, Bruschettini, and others have obtained from the blood cultures of what they believed to be Pfeiffer's bacillus. Successful cultures are sometimes found to have been secured from blood squeezed from the finger-tip, as by Slawyk.

Pfeiffer and Kruse and others were unable to isolate the bacillus of influenza from the blood, and denied the identity of some of the germs obtained from the blood by others.

Recently Kuhnau has added twelve negative results, and there seems little reason to doubt that the bacillus of influenza has no special capacity to invade the blood stream.

RABIES.

Friedrich,[2] in 1869, observed an increase of white blood cells eighteen hours before death.

Courmont and Lesieur have recently followed the leucocytes in the disease in both man and animals. Rabies in man is apparently unaccompanied by leucocytosis until a few hours before death, when a terminal increase, principally of polynuclear cells, appears.

In two cases one hour before death 21,000 and 24,000 cells were counted, while in three cases the antemortem proportion of polynuclear cells was 83 to 88 per cent. In experimental rabies no leucocytosis developed until nervous symptoms appeared.

In both man and animals, however, an excess of polynuclear cells was found before any distinct increase in their numbers. In spontaneous rabies in dogs (five cases) there was leucocytosis, with 88 to 98 per cent. of polynuclear cells, twenty-four to forty-eight hours before death.

TETANUS.

Cabot reports 70 per cent. of Hb, 11,900 leucoc[illegible]d persistence of eosinophile cells, in a fatal case of tetanus t[illegible]itoxin, and 19,600 in a second case.

BUBONIC PLAGUE.

Coagulation of the blood failed entirely in ten of twelve fatal cases in which the blood was collected in sealed tubes by Corthorn, while in two other fatal cases the clot was imperfect.

The Austrian Pest Commission found 65 to 80 per cent. Hb in the majorty of cases. Leucocytosis was always present, but not in excessive degree, the numbers varying between 12,000 and 28,000.

Aoyama, in 1895, examined the blood of many cases, reporting four in full. The red cells varied from 4,400,000 to 8,100,000, the latter in a case which recovered, and in the report of which no cause of polycythemia is apparent. The Hb was normal. The leucocytosis also was reported to have reached a very high grade. Polynuclear cells made up the bulk of the increase, but large and small lymphoocytes were numerous, and eosinophiles scarce.

Zabolotny studied the reaction of the blood serum on cultures of the bacillus. During the first week no effect was observed even with a dilution of 1 : 6; in the second week reactions were obtained with dilutions of 1 : 10; in the third week with 1 : 25; in the fourth week with 1 : 50. The reaction was most marked in the severer cases, and the blood serum of the cadaver was inert.

Kitasato, in 1894, reported the demonstration of *bacillus pestis* in twenty-five of twenty-eight cases in smears of blood squeezed from the finger-tip, but this method gives no reliable indication of the presence of the organism in the circulating blood. Many later observers, relying on the same method, have drawn erroneous conclusions regarding the occurrence of this bacillus in the blood. Thus, Rees found it in the smears of the blood when cultures of the blood drawn aseptically were sterile. The Austrian Commission and others came to the conclusion that the bacteriological examination of blood smears in plague is not only unreliable, but fallacious and mischievous. They placed little diagnostic value even on the proper bacteriological examinations of the blood.

MALTA FEVER.

The hemorrhages which mark the severer forms of this malady commonly give rise to severe anemia. Bruce places the average number of red cells at 3,500,000, and reports the absence of leucocytosis and occasional presence of free pigment in the blood. In a prolonged case without hemorrhages Musser and Sailer found 5,050,000 red cells, 60 per cent. Hb, 11,564 leucocytes, all varieties being in normal proportions.

Wright and Smith found that the blood serum of cases of Malta fever agglutinates cultures of *bacillus melitensis*, and diagnoses of the disease have been made by this method by Musser and Sailer, Cox, and others. A dilution of serum 1 : 50 should give a prompt reaction, while typhoid serum has no effect.

ACTINOMYCOSIS.

The writer found 21,500 leucocytes in a case of pulmonary actinomycosis, clinically resembling acute phthisis, and Cabot reports a case of actinomycosis of liver with 3700 white cells.

The markedly purulent character of the exudate excited by the ray fungus explains the grade and character of the leucocytosis. Bierfreund found marked chlorotic anemia in actinomycosis, the Hb registering 30 to 50 per cent.

GLANDERS.

Cabot refers to a case of acute glanders in which the fibrin was increased, the Hb normal, and the leucocytes varied between 11,600 and 13,600, 86 per cent. of which were polynuclear. Coleman and the writer reported an acute case with extensive pulmonary lesions, in which the leucocytes steadily numbered 13,000. *Bacillus mallei* was isolated from the blood three days before death.

Duval isolated the bacillus of glanders from the blood during life, and Noniewitch reports that in fatal cases in horses the bacilli can be found in the circulating blood, usually within the leucocytes.

ANTHRAX.

Anthrax in some lower animals frequently develops into a pronounced bacteremia early in the disease. Man, however, usually exhibits only a moderate grade of susceptibility to the infection and the majority of cases recover after excision of local foci of infection. Although it is probable that in the severe septicemic forms of the disease anthrax bacilli may multiply in the general blood stream early in the disease, the literature appears to contain very few reports of competent bacteriological analyses of the blood in such conditions. The rather frequent impression that the diagnosis of anthrax can be made by morphological examination of a drop of blood from the general circulation is almost entirely without foundation, as the great majority of the cultures of the blood, even in severe cases, have been found sterile. Only in the late stages of some septicemic cases in man do the bacteria invade the circulation and yield positive cultures of the blood. Such a case is that of Blumer and Young, who report successful cultures, and claim to have identified the germ in blood smears during life, but the time before death was not stated.

Antemortem invasion of the blood by anthrax in man is apparently not much if any more abundant than with some other infections, and even postmortem cultures are frequently negative.

Detailed clinical examinations of the red cells and leucocytes in the blood of anthrax are still wanting.

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CHAPTER XVI.

SYPHILIS, TUBERCULOSIS, LEPROSY.

SYPHILIS.

VERY accurate conclusions regarding the state of the blood in syphilis were reached by Becquerel and Rodier, who stated that a moderate grade of anemia were to be found in the great majority of cases, that as long as the disease progressed without complications the blood maintained a high or normal standard, and that if the course of the disease were prolonged there was a loss of red cells. They noted also that the abuse of mercury might lead to the same changes. The resemblance of the anemia of syphilis to that of chlorosis was also early recognized, especially by Ricord, and this belief was later supported by analyses of the blood by Grassi, Wilbouchewitch, Keyes, Laache, Malassez,[1] Gaillard, and others, who found in the secondary and tertiary stages of most cases a loss of red cells and Hb. Keyes was the first to point out that mercury in small doses, while curing the disease, increases the red cells.

The *grade of anemia* observed was usually moderate, the majority of cases showing slight loss of red cells, which, however, sometimes fell to 3,000,000 to 4,000,000. The Hb was usually found diminished more than the cells, especially by Lezius, who claimed that the only essential lesion of the blood in syphilis is a loss of Hb. Other patients appeared to have *normal blood*, and Sorensen in twenty cases failed to detect any distinct anemia. On the other hand, Müller reported cases in which all the typical lesions of *pernicious anemia* were present, and in one of his cases the red cells fell to 428,100. Other such cases are reported by Ponfick, Kjerner, Klein, Laache, Fisischella (cited by Dominici).

It was thus established that syphilis in some stages and in some patients may entirely fail to reduce the red cells, while in others grave pernicious anemia must be charged to its action. The later studies have been concerned with the more detailed course of the blood changes in the disease, and have been contributed principally by Ane, Lezius, Bieganski, Konried, Rille, Loos, Justus, and Riess.

From these contributions it appears that the blood suffers in a somewhat uniform degree in the several stages of uncomplicated syphilis, but unfavorable conditions and abuse of mercury may greatly aggravate the changes observed.

Primary Stage. During the first four to seven weeks after infection the red cells do not diminish perceptibly in number (Lezius, Konried, Oppenheim and Lowenbach) unless there is fever or some other disturbing factor, when a moderate decrease may be noted from the first (Stoukovenkoff). No doubt, as Hayem says, these disturbing factors are frequently present, and it still remains uncertain whether syphilis alone affects the number of red cells during the first few weeks. The majority of observations indicate that it does not, but Riess came to a contrary conclusion, while Bieganski found uniform polycythemia in the early stages of syphilis. Recently Oppenheim and Lowenbach in thirty-four cases failed to find any distinct reduction in red cells.

The Hb is almost invariably diminished from the first, a loss of 15 to 30 per cent. being commonly noted before the appearance of secondaries (Konried).

Secondary Stage. There is uniform agreement among very numerous observers that with the outbreak of secondary symptoms the red cells begin to fall rapidly, reaching in untreated cases as low as 2,000,000 or less (Konried). In ten cases Wilbouchewitch found an average decrease of 229,000 cells daily. At the same time the Hb continues to diminish and may fall to 55 or 25 per cent. within a few weeks or months. Riess denies that the Hb suffers particularly at the outbreak of the eruption. With the appearance of fever and new eruptions a further and more marked loss of red cells and Hb has been observed (Stoukovenkoff). Under favorable hygienic conditions and in feeble and especially in young subjects the anemia in this stage may be unusually severe. In untreated cases the disappearance of the eruption is not followed by any immediate improvement in the blood.

Tertiary Stage. In untreated cases there can be no doubt that the anemia may progress until the pernicious type is established, but treatment usually limits the impoverishment of the blood, so that only moderate grades of anemia are commonly observed. Konried found an average of 4,000,000 red cells in ten cases, and from 50 to 80 per cent. of Hb in twenty-two cases suffering from gummatous lesions.

In several cases showing advanced tertiary lesions (gummata) at autopsy the writer has found different grades and types of secondary chlorotic and pernicious anemia. In one the spleen was much enlarged and contained gummata, while the blood showed the lesion of secondary pernicious anemia, with a tendency toward the microcytic type, and with low Hb-index. In other cases the abundant megalocytes with increased Hb closely resembled those of primary pernicious anemia. It is especially in infants that syphilis induces the grave types of anemia.

Effects of Mercury upon the Blood in Syphilis. The effect of proper doses of mercury in arresting the progress of the anemia of syphilis was clearly stated by Wilbouchewitch, who reported an average gain of 102,000 cells daily in ten cases, while Keyes and many later observers have fully verified these results. That prolonged use of mercury may of itself lead to marked anemia resembling that of syphilis was early noted by Becquerel and Rodier, and was also demonstrated both in man and animals by Wilbouchewitch, and later by Bieganski, Hayem, Lezius, Anc, Schlesinger, and

Jelleneff. The extent to which mercurial treatment may be carried without diminishing the red cells or Hb has been placed at twenty-four days, by Gaillard; at twenty-five to thirty-five inunctions, by Konried; at sixteen injections (grammes 0.5, 1 per cent. Hg-benzoate) by Jelleneff; and at 140 to 150 milligrammes of bichloride or 77 milligrammes of benzoate, injected in increasing doses, by Lindstrom.

During this period most observers agree that both red cells and Hb increase from the first, and many instances of moderate polycythemia are recorded. At the same time the eruption commonly disappears. If treatment is continued beyond this point, especially if the patient is salivated, the cells and Hb steadily and sometimes rapidly decline and well-marked mercurial anemia is established. Jawein, however, failed to observe any anemia during prolonged courses of inunctions.

The Leucocytes in Syphilis. Leucocytosis was early observed in syphilis and its usual connection with the hyperplasia of lymph nodes was one of the facts that led Virchow to locate the origin of the lymphocytes in these structures. More detailed study of the behavior of the leucocytes was made by Wilbouchewitch, Bieganski, Jelleneff, Konried, and Rille, who have shown that the leucocytosis of syphilis is connected principally with the eruptions and the anemia of the disease.

In the primary stage, before the appearance of the eruption, the leucocytes are usually normal (Rille), but Jelleneff found that an increase of white cells usually precedes the development of anemia, and Konried's cases showed a slight leucocytosis (maximum, 16,400) in the first weeks of the primary stage.

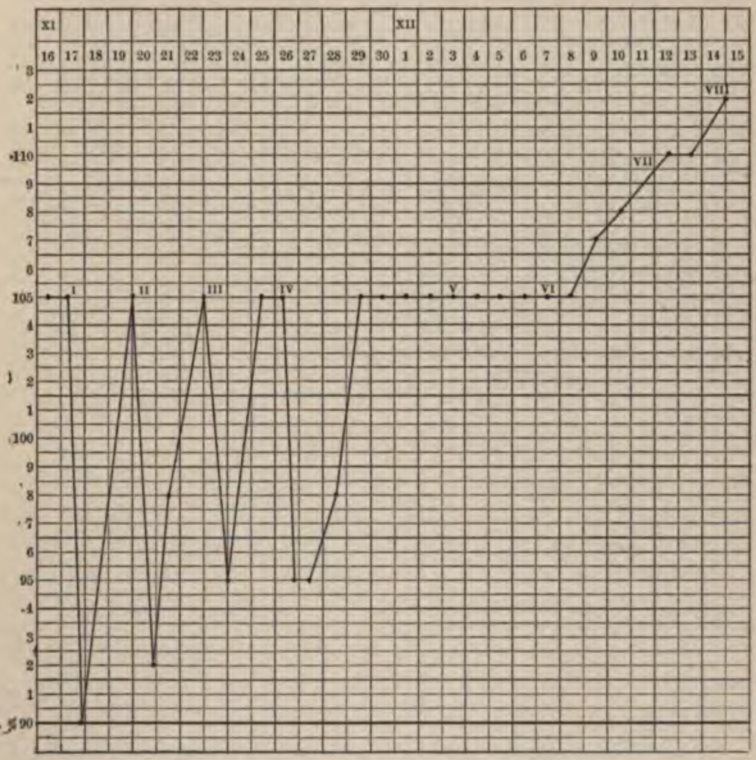
In the secondary stage, with the appearance of eruptions, anemia, and hyperplasia of lymph nodes, the leucocytes are nearly always increased. In Konried's cases of untreated secondary syphilis the white cells were never under 10,000, while the maximum figure was 17,500. Riess, however, found leucocytosis absent in many cases, but observed an increase to 20,000 in some instances, while the excess of lymphocytes was usually very distinct (maximum, 68 per cent.). The administration of mercury commonly reduces the white cells, which become normal with the disappearance of the eruption and the anemia. Jelleneff found the leucocytosis to be more nearly proportionate to the extent of the eruption than to the size of the lymph nodes, and noted leucocytosis in the absence of external signs of lymphoid hyperplasia.

The increase affects principally the small and large lymphocytes, but the eosinophile cells may also be increased, especially in those cases with marked papular exanthems (Rille). Zappert found a slight increase of eosinophiles, 4.91 per cent., in one early case, but normal numbers in seven others. The proportion of lymphocytes diminishes as the patient improves, falling after each inunction (Riess). In very severe cases there may be progressive polynuclear leucocytosis. Myelocytes have been found by Rille and others in both secondary and tertiary syphilis.

In tertiary stages with gummatous lesions the leucocytosis usually persists, but lymphocytosis, though sometimes distinct, is less constant. Konried found 8500 to 17,710 leucocytes in nine cases.

Justus' Test in the Blood of Syphilis. By a series of observations in over 300 cases Justus came to the conclusion that in the blood of florid syphilis after injections of moderate doses of various preparations of mercury, or after inunctions, there is a period of a few hours or days during which the Hb is considerably reduced (10 to 20 per cent.). After a certain period, varying with the general condition of the patient and the severity of the symptoms, the Hb begins to increase. In some cases the diminution continued only one day and in others it was repeated after each of three or four injections. The reaction was noted in all stages of the disease after the primary swelling of lymph nodes, and in hereditary syphilis, but could not be obtained "during or shortly before the symptoms of the disease begin to disappear."

FIG. 35.



Course of changes in Hb in Justus' test. (Roman numerals indicate intravenous injections of sublimate.)

In the serum, within two minutes after the intravenous injection of bichloride, Justus found the spectrum of oxyhemoglobin. In blood smears immediately after the injection many pale degenerating red cells were found, which disappeared within a few minutes. It thus appeared that the mercury had caused an immediate solution of red cells in the plasma. The specific gravity of the blood was not studied except in one case in which it showed irregular fluctuations.

Justus' test has been applied in 121 cases, collected from the reports of Cabot, Jones, Christian and Foerster, Brown and Dale, Huger, Oppenheim and Lowenbach, and Tucker. In active syphilis it was positive in 46 cases, negative in 15, and the Hb rose instead of falling in 13. In cases of chancre there are 11 positive and 20 negative results, and in chancroid 6 positive and 3 negative tests. Three negative results with herpes, 2 positive results in genito-urinary tuberculosis, and 1 positive result in chlorosis and in sexual neurasthenia complete the observations of the above writers.

With only 62 per cent. of positive results in active syphilis, 30 per cent. in chancre, and 67 per cent. in chancroid, it is evident that Justus' test can have only a limited value in the diagnosis of syphilis.

Lately, however, Justus has reiterated his belief that the test is a specific sign of florid syphilis. He explains the negative results of other observers as due to the use of too little mercury or the absence of lesions of the lymph nodes as a necessary sign of a florid stage of the disease. Only inunctions of ung. hydrarg. he now finds suitable for the test, and three grammes must be used with adults or one gramme with children.

Further studies are required before the value of Justus' test can be shown, but on general grounds it would seem that the test would prove unreliable in many forms of anemia, when the globulicidal action of mercury might dissolve red cells.

Congenital Syphilis.

Syphilitic infants invariably suffer from anemia. In the mildest cases Loos found over 5,000,000 red cells and 65 to 75 per cent. of Hb. Schiff also found the usual polycythemia of infants in mild cases of congenital syphilis. In the majority of cases there is considerable loss of red cells and the Hb falls to a low figure, minimum 21 per cent. (Loos). In the severer forms the blood may show the changes of secondary or progressive pernicious anemia, examples of which have been described by Loos, Luzet, Monti, Berggrun, and others.

Whatever the grade of anemia, the changes are influenced by the special tendencies of infants' blood. Marked differences in the size of the cells are early established, nucleated red cells are frequently seen in abundance, and white cells, *especially lymphocytes*, are often present in greatly increased numbers. Loos noted an extreme degree of polychromasia in the large nucleated red cells in congenital syphilis. The leucocytes did not fall below 12,000 in any of Loos' sixteen cases, and in one fatal case rose to 58,000. Baginsky, and Monti and Berggrun have also reported excessive leucocytosis in similar cases, while the former author speaks of a distinct leukemic tendency to be found in the blood of congenital syphilis.

The *lymphocytes* usually fall within normal limits for this age. *Eosinophile cells* are sometimes increased, especially when the eruption is extensive (Loos). The presence of a few *myelocytes* in the severe cases makes the resemblance of these cases of syphilitic anemia to v. Jaksch's anemia often very close, while the excess of large lymphocytes may lead to confusion of the disease with lymphatic leukemia of Fraenkel's type.

Congenital syphilis is recognized by Demelin as one of the common causes of *melena neonatorum.* In such a case, dying four days after birth from abdominal hemorrhage, the writer found in the blood the typical characters of idiopathic pernicious anemia, especially in the large number of megaloblasts. The marrow was everywhere lymphoid, and showed pronounced megaloblastic changes.

TUBERCULOSIS.

The fact that the blood of consumptives may often fail to show any changes comparable with the pallor of the skin and the emaciation of these subjects was noted in the first studies of the blood by Andral and Gavarret, Becquerel and Rodier, and others. The discrepancy between blood and facial appearance was fully recognized by Laache, who, finding an average of 4,400,000 cells in fourteen cases, stated that phthisis of itself usually does not tend toward marked anemia. Similar results in the hands of Sorensen, Oppenheimer, Gnezda, Barbacci, and Reinert, confirmed this opinion in a considerable group of cases, although well-marked chlorotic anemia was occasionally encountered. In the Massachusetts General Hospital series Cabot found forty-one out of sixty patients with more than 4,000,000 red cells, and sixty-nine of eighty cases with more than 50 per cent. of Hb.

Yet Malassez had in 1874 reported well-marked anemia in some early cases, had seen the red cells fall over half a million in the course of one week, and found less than a million cells in an advanced case with diarrhea.

In nearly all of their series of cases Laker and Fenoglio found low percentages of Hb, and the specific gravity was found markedly reduced by Schmaltz and Peiper.

In 1893 current views were presented by v. Noorden, who stated that in pulmonary tuberculosis a loss of more than 20 per cent. of red cells or Hb is seldom observed unless from complications such as hemorrhage, suppuration, or amyloid degeneration.

The subject was at this time thoroughly reviewed by Grawitz[1] and Strauer, who found three distinct periods in the changes of the blood in phthisis:

1. With beginning apical lesions, they generally found marked chlorotic anemia with loss of red cells, irregular leucocytosis, and reduction in dry residue and specific gravity. In robust subjects, however, the blood during this stage was sometimes normal.

2. In pale, emaciated subjects with chronic phthisis and cavities, but without much fever, the blood commonly did not vary from the normal standard, the red cells numbering 5,000,000 or [illegible] the leucocytes 5000 to 10,000, the dry residue of blood and [illegible]

moderately low, but the specific gravity higher than the dry residue would indicate.

3. When hectic fever supervened, usually from suppuration in cavities, and the patient began to suffer from septicemia, the blood became rapidly impoverished, the red cells were then much reduced, the leucocytes frequently much increased, and the dry residue and specific gravity falling to a low point.

The intense septicemia of rapidly advancing phthisis was found to yield some very severe grades of anemia, in one of which the red cells fell to 700,000. Similar cases have been reported by Malassez[2] and Limbeck,[1] both of which suffered from marked diarrhea. In Limbeck's case there was also tuberculous peritonitis, and the blood showed marked poikilocytosis and increased Hb-index.

In the attempt to correlate his own and Kobert's divergent results, Dehio described two different types of the disease, the *marantic* and the *anemic* cases. In the former group the patients showed a marked tendency to emaciate while their blood retained a good standard, while in the others all grades of chlorotic anemia might be observed.

It would seem that the classifications of both Dehio and Grawitz are based on accurate clinical study. The following conclusions may be drawn regarding the general course of blood changes in tuberculosis:

1. *The primary anemia of tuberculosis* is seen not only in diseases of the lungs, but quite as frequently in chronic tuberculosis of other tissues, especially of the lymph nodes and bones, constituting the very numerous group of cases of "*lymphatic anemia.*" Moreover, it has recently been placed beyond doubt that the chronic anemia of Hodgkin's disease is in a large proportion of cases the result of tuberculosis of lymph nodes.

The peculiar character of these cases of secondary tuberculous anemia has been described by Laker, Neubert, Wiskeman, Vierordt, Bierfreund, Brown, Dane, and many others, and they constitute one of the largest groups of cases encountered in routine blood examinations. In appearance the patients clearly resemble cases of chlorosis and the blood shows a slightly or considerably reduced number of red cells, more marked loss of Hb, and usually slight relative or absolute lymphocytosis. Yet this description does not apply to all cases of early tuberculosis or phthisis, as some of them from the first appear anemic and yet show normal cells and Hb.

2. The second group *in which the blood is but slightly altered in quality*, although cavities have formed in the lung and the patient is pale and emaciated, includes the majority of cases of chronic phthisis. One of the most striking examples seen by the writer occurred in a patient suffering from subacute tuberculous empyema from which the pus contained very large numbers of tubercle bacilli. But the same quality of blood is sometimes seen with early apical lesions and moderate emaciation, while considerable anemia may exist in moderately advanced cases of phthisis who are *not* suffering from distinct hectic fever.

The cause of the concentration of the blood, or oligemia, in the average phthisical subject, for such must be the condition, is probably to be found, as indicated by Heidenhain, Gartner and Romer, and Grawitz,[2] in the specific lymphogogic action of the toxins of the tubercle bacillus, by which there is established a continuous excess in the balance of fluids which leave the tissues through the lymphatics. This view is strongly supported by the resemblance which exists between the tuberculous and the typhoidal processes, both showing a peculiar relation to lymphatic structures and both tending to concentrate the blood. Identical effects upon the blood follow also the experimental injection of tuberculin and typhotoxin.

By this means the considerable destruction of blood which results from the chronic toxemia, hemorrhages, and malnutrition of the disease is obscured, and only becomes visible at autopsies on these subjects when the shrunken appearance of the tissues finds a parallel in the diminished total volume of blood which is often apparent to the naked eye. *In the majority of cases of well-advanced phthisis, therefore, approximately normal blood indicates considerable absorption of the toxins of the tubercle bacillus.* Prolonged and profuse night-sweats, and severe diarrhea, doubtless have a similar effect, which, however, is usually overbalanced by the destruction of blood which results from the associated toxemia. Normal blood is found in many phthisical patients who do not sweat or suffer from diarrhea. Yet in some extreme cases one or both of these factors may very well prove to be the chief influence in the concentration of the blood.

3. The third group of cases includes those who are anemic from the first or who become anemic in the terminal stage of the disease, in either instance from the destruction of blood which occurs in all severe toxemias. Since the severe hectic fever of phthisis is largely referable to mixed infection, the condition established is not very different from the ordinary type of pyogenic sepsis. The destruction of cells may be quite rapid. Malassez observed losses of 730,000 cells in one month, and 760,000 in three weeks in cases without hemorrhage. Here belong the acute cases with grave anemia described by Malassez, Limbeck, and Grawitz, from which it appears that the blood in tuberculosis may develop much the same characters as in *pernicious anemia.* Hills reports a chronic case of general tuberculosis with melancholia, in which the red cells fell to 155,000.

Morphological changes in the red cells are rather less marked than in most other types of secondary anemia, owing probably to the conservative effect of a plasma of high gravity. When anemia exists it is usually of the simple chlorotic type with relatively high Hb-index. In his case of grave anemia Limbeck described extreme poikilocytosis, but nucleated red cells were apparently absent. In two very anemic acute cases lasting four and five weeks, the writer found under 2,000,000 red cells, but there were no distinct megalocytes and no nucleated red cells. Cabot finds that nucleated red cells are usually absent in tuberculosis, even after hemorrhage, and contrasts this fact with their abundance in carcinoma. Degenerative changes in the red cells in tuberculosis are not marked. In thirteen cases of uncomplicated phthisis Grawitz[3] found no signs of granular degeneration.

Effects of Complications. *Hemoptysis* causes impoverishment of the blood in proportion to the extent of the hemorrhage. Malassez observed a reduction of 940,000 cells from small hemorrhages in a period of eight days. *Amyloid degeneration* is seen with advanced and anemic cases, but its effects on the blood have not been specially studied. Stenosis of the larynx and diabetes have been found with concentrated blood (Grawitz).

Regeneration of the Blood in Phthisis. Prompt regeneration of the blood after hemoptyses has been observed by Malassez, but such improvement must depend largely upon the general condition of the patient. Laker came to the conclusion that if the Hb did not steadily improve after operation on tuberculous foci, it might safely be concluded that all of the disease had not been removed. In seven of Bierfreund's cases and in two of Brown's this rule held good, and three times a steady decline in Hb preceded for several weeks the development of symptoms of general tuberculosis (*vide infra*).

In phthisis as well as in other tuberculous processes great caution must be used in judging of the patient's improvement from an increase in red cells or Hb. The writer has seen the Hb and red cells increase while the patient was rapidly losing flesh, the lesions advancing, and the total quantity of blood doubtless falling. In several of Bierfreund's cases the Hb steadily increased while the patient was developing general tuberculosis.

The Leucocytes in Tuberculosis. In the majority of cases of uncomplicated tuberculosis the leucocytes remain within normal limits or are distinctly deficient in number. This rule has been established principally by later observers, since the earlier studies cited by Rieder, Reinert, and others indicated that tuberculous inflammation usually excites leucocytosis.

Yet Halla noted that leucocytosis was usually found only in advanced cases with fever, and Rieder distinguished the fresh cases from chronic febrile tuberculosis by the absence of any increase of white cells in the former. It is perhaps a still more uniform rule that leucocytosis is found almost exclusively in the febrile and anemic cases, but it is absent also in many febrile periods.

In pulmonary tuberculosis an increase of leucocytes is usually referable to *suppurating cavities, advancing pneumonia, severe anemia,* or *hemoptysis. Suppurating cavities,* when of recent formation, usually raise the leucocytes distinctly, 15,000 cells being frequently seen, and when the lung is softening the excess may be much greater. The leucocytosis usually persists while the fever continues. With old cavities the expectoration may be abundant, but the leucocytes usually fall to normal.

In the *pneumonia* of various types which complicates phthisis the leucocytes are usually much increased, but considerable areas of diffuse tuberculous pneumonia may be found with acute miliary tuberculosis, which have failed to increase the leucocytes during life. A lobar pneumonia causes the usual leucocytosis, but the writer has seen both lungs consolidated and riddled with small cavities, in a case lasting five weeks, yet the leucocytes were never found above 12,000. *The absence of leucocytosis in these cases of acute phthisis which resemble pneumonia may often be of value in diagnosis.* Similarly in a case of subacute empyema in which the tubercle bacillus was largely concerned, the leucocytes were found not to exceed 14,000 during an acute febrile period.

The leucocytosis of *tuberculous cachexia* is moderate, usually between 10,000 and 15,000. After *hemoptysis* the leucocytes are increased in proportion to the hemorrhage, but the increase is transient.

Acute general miliary tuberculosis offers the best illustration of the failure of pure tuberculous inflammation to induce leucocytosis. Rieder, Limbeck, Warthin, and Cabot find rather a diminution of leucocytes, often to an extreme degree, and, as Warthin has shown, during a prolonged period. Minor suppurative complications seem to have no capacity to raise the white cells, but in some reported cases a high percentage of polynuclear cells was noted among the scanty leucocytes (Warthin).

Tuberculous inflammations of serous membranes, when uncomplicated by pyogenic infection, follow the type of pure tuberculous inflammation elsewhere and fail to increase the leucocytes. In many cases, however, there appears to be mixed infection, and the effusion, instead of remaining serous or serosanguinolent, becomes seropurulent and purulent. In such cases of pleurisy with seropurulent or purulent exudate the writer has found a moderate increase of leucocytes, usually in proportion to the height of the temperature. While it is undoubtedly true that the tubercle bacillus may of itself produce pus, the fact remains that it is seldom or never seen alone in purulent exudates. The slight leucocytosis that accompanies cases of purulent tuberculous inflammations of serous membranes is perhaps a further indication that these cases represent mixed infections.

Tuberculous Meningitis. In the majority of reported cases of this important localization of the disease leucocytosis has been absent, as seen by Limbeck,[2] Pick, Rieder, Sorensen. Yet in five of seven cases reported by Cabot and in one by Ziemke there was distinct leucocytosis, from 14,700 to 34,300, while in one of Rieder's cases there were 14,400 white cells. It is difficult to reconcile these conflicting results. Some of the leucocytoses may have been ante-mortem phenomena, but not all. Ziemke's case was one of advanced general tuberculosis. The writer has seen cases with leucocytosis, but complicating terminal pneumonia was in each instance found at autopsy.

Morphology of Leucocytes in Tuberculosis. In most cases of chronic tuberculosis, when the leucocytes are normal or diminished, the mononuclear cells are relatively in excess, but in some instances the usual rule is reversed, as in Warthin's case, and the polynuclear cells are in excess. Distinct lymphocytosis is seen in many chronic cases, especially those with large lymph nodes (lymphatic anemia). Lymphatic leukemia sometimes develops in tuberculous subjects, but the relation of the two diseases is uncertain. When distinct leucocytosis occurs, excess of polynuclear cells is usual, and pyogenic infections, etc., are indicated.

Neusser believes that eosinophilia indicates relative immunity to tuberculosis. General experience seems to show that eosinophiles are frequently present, usually in normal numbers, in the blood in chronic tuberculosis, and sometimes absent in the acute cases, or when there is fever and leucocytosis. Zappert found none in 2 of 5 cases of febrile tuberculosis. They are commonly increased, sometimes to an extreme degree, in the febrile period following injections of tuberculin. (Zappert.)

Tuberculosis of Bones and Joints. Valuable studies in this field have been contributed by Brown in seventy-two and Dane in forty-one cases of tuberculosis of hip and spine.

From these studies it appears that the above conditions are very frequently accompanied by moderate or marked leucocytosis (maximum, 41,000), which is usually referable to secondary infection and the formation of pus. Yet Brown observed several cases with leucocytosis but without formation of abscess, and believes that leucocytosis may be referable alone to increased activity of the tuberculous process. A frequent cause of leucocytosis, often of marked degree, was secondary infection of the sinus after operation. The leucocytosis was usually but not always associated with fever. Both authors agreed that when leucocytosis was absent in cases with abscess secondary infection had not occurred, since the pus was sterile. They did not apparently consider the possibility that a micro-organism once present may disappear, or that leucocytosis may disappear when pus ceases to form.

Myelocytes have been reported in scanty numbers in a few cases.

Chemistry. Special chemical alterations of the blood in tuberculosis have not been demonstrated, but the usual changes of secondary anemia have been reported in a few cases. Hammarschlag found a low gravity only in cachectic cases. Moderate reductions only were observed by Devoto and Scholkoff, but Schmaltz found a gravity of 1.036 in an advanced case of phthisis, and Grawitz's case of grave anemia gave 1.032.

The gravity is much lower, in proportion to the loss of cells, than in pernicious anemia. Dieballa found a density of 1.039 in a case of phthisis with 2,400,000 cells, and in one of pernicious anemia with only 840,000 cells. Mircoli found the serum actively hemolytic in the early stages of some cases.

Freund found in the blood of tuberculous subjects a body which he regarded as cellulose, which Nischimura has likewise identified, while others have found the same substance in tuberculous tissues. It is probably derived from the bodies of the tubercle bacilli.

Bacteriological Examination of the Blood in Tuberculosis. Even before the discovery of the tubercle bacillus Weigert had pointed out the considerable involvement which the vessels may show in acute miliary tuberculosis. Weichselbaum, in 1884, was able with prolonged searching to find numbers of tubercle bacilli in stained specimens of the clotted blood of the great vessels in three cases of acute general tuberculosis. In the same year Meisels and Lustig both succeeded in finding tubercle bacilli in the blood *intra vitam*, the specimens having been squeezed from the finger-tip during a period of rising pyrexia. In the blood of the spleen the bacilli were much more numerous and Meisels recommended *aspiration of the spleen as an aid in the diagnosis of obscure cases.* Rutimeyer also succeeded in finding many bacilli in the aspirated splenic blood of one of two cases examined shortly before death. In a case which eventually recovered Sticker, after considerable search, found a few bacilli in the finger blood taken on the tenth day of the illness.

With the introduction of tuberculin, Liebman claimed to have found tubercle bacilli in 56 of 141 blood specimens, most abundantly about twenty-four hours after the injection of tuberculin. Grave doubt was, however, thrown upon all previous work in this field by the negative results obtained by Guttmann, and by Kossel, who called attention to the danger of contamination in such specimens. This method of diagnosis has not been followed up in later years, although Kronig, in 1894, recommended the staining of centrifuged laked blood in doubtful cases of miliary tuberculosis.

In the blood of tuberculous cadavers various micro-organisms have been isolated by Pasquale, Petruschky, Canon, Welch and Nuttall, and others. Cultures of the blood during life have been made, by reliable methods, by Sittmann, Kraus, Kuhnau, Hewelke, White, Michaelis and Meyer, and Hirschlaff.

Of their seventy-nine cases of advanced and usually febrile phthisis, there were twenty-three positive and fifty-six negative results. *Staphylococcus pyogenes aureus* or *albus* was isolated in twenty cases; *streptococcus pyogenes* and a diplococcus in one case each, and the tubercle bacillus was once obtained from inoculation by Kuhnau. The pyogenic germs were sometimes found together. Jakowsky and Petruschky were rather more successful, drawing the blood through the skin, but this method is unreliable.

There can be little doubt, therefore, that the blood in the late stages of phthisis suffers bacterial invasion as in other forms of septicemia. Kuhnau's one positive result from twelve inoculations of the blood of severe febrile cases does not encourage further search for the tubercle bacillus in the blood of tuberculous subjects. Lasker also, in sixty-eight cases of hectic tuberculosis, found bacteria in the blood, streptococci but once.

LEPROSY.

Winiarski, in 1892, studied the blood in seventeen cases illustrating various phases of this disease. In some cases he found polycythemia, 6,380,000 to 6,090,000 red cells, which may perhaps be referred to local stasis, as the hands appeared cyanosed. Few of the patients showed any marked loss of cells, the average being 5,050,000 for nine men, and 4,300,000 for eight women. Winiarski, therefore, concluded that the milder cases of one or two years' standing do not suffer from any distinct anemia. Yet in two advanced cases with extensive lesions the red cells numbered 2,300,000 and 1,900,000, and the latter patient presented many signs of pernicious anemia.

In these cases the anemia was referred to extensive ulceration. The Hb suffered even less than the cells, usually registering 80 to 118 per cent. in all but the two very anemic cases. The Hb-index was uniformly high, and in one case with 1,900,000 cells and 64 per cent. Hb the index reached 1.7. An increased diameter of the red cells was noted in this and other cases. The *leucocytes* were normal or subnormal in number, and the lymphocytes were in relative excess (maximum, 47 per cent.), except when suppuration occurred.

Brown, more recently, reported his observations in sixteen cases, eight of which appeared to be quiescent and presented normal blood, while only one showed severe anemia. He claimed to have found the bacillus in the leucocytes of the circulating blood in nine cases, eight of which showed the tubercular type of lesion.

Streker also examined the blood of five "very anemic" cases of leprosy and found bacilli both free in the plasma and inclosed in leucocytes. The blood was drawn with antiseptic precautions from a deep incision through normal skin. These observations are quite in accord with the reports of Joseph regarding the large deposits of *bacillus lepræ* in the spleen.

Sprouck claims that the serum of leprous subjects, in dilutions between 1 : 60 and 1 : 1000, agglutinates fresh living cultures of the bacillus of Hansen, and he recommends the use of this test in diagnosis.

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PART IV.

CONSTITUTIONAL DISEASES.

CHAPTER XVII.

HEMORRHAGIC DISEASES, THE HEMORRHAGIC DIATHESIS, AND INTOXICATIONS.

HEMORRHAGES of greater or less extent occur in many general diseases, in which the loss of blood is not referable to any specific predisposition on the part of the individual, but to peculiar conditions arising in the course of the disease. Such hemorrhages are regarded as *symptomatic*.

The chief causes of symptomatic hemorrhages are:

1. Infections, as in any severe infectious diseases, such as septicemia, scarlatina, smallpox, measles, etc.
2. Mechanical, as in partly asphyxiated infants, or other conditions of marked venous stasis.
3. Toxic and autotoxic, as in poisoning by the so-called blood-poisons, or in jaundice, anemia, etc.
4. Nervous, as in rare forms of hysteria.

In the hemorrhagic diathesis probably all the above factors are at times unusually active, but there is believed to be in addition a specific, pre-existing, often hereditary change in the blood which largely determines the various manifestations of this diathesis. Modern bacteriological and chemical research has now considerably narrowed the scope of the hemorrhagic diathesis, and at present it is impossible to claim that any peculiar predisposition to hemorrhage exists except in cases of *hemophilia*.

Yet it remains convenient to describe the various idiopathic purpuras, scurvy, and hemophilia as closely related conditions.

PURPURA HEMORRHAGICA. (WERLHOFF'S DISEASE.)

The etiology of this disease still remains obscure, although the evidence points more and more toward some form of infection as the essential element in the majority of cases. The prominence of hemorrhage in the symptoms of many infectious diseases has prepared the way for an acceptance of this view. In adults the extent of the hemorrhage attending well-identified forms of infection is rarely so great as in pronounced cases of *purpura hemorrhagica*, in which of no bacterial origin has been demonstrable. In infants, however, infections by the ordinary pyogenic bacteria have become recognized as a frequent source of fatal hemorrhages constituting for that age the typical picture of Werlhoff's disease.

There are now many cases of purpura hemorrhagica on record in which bacteriological examination of the blood or viscera showed the presence of bacteria, which were regarded as the cause of the disease. In some cases bacilli were isolated, which were not fully identified. Letzerich's *bacillus purpuræ* in many respects resembled *bacillus anthracis*, and produced in animals, and possibly in the investigator's own person, a hemorrhagic disease resembling Werlhoff's. *Streptococcus pyogenes* has been isolated in typical cases by Hanot and Luzet, Widal and Therese, Guarnieri, and others. In the first of these the disease was transmitted from mother to fetus, the latter dying and yielding a pure culture from the blood. In infants, rapidly fatal hemorrhagic infections of this nature are not rare.

Staphylococcus pyogenes aureus has been isolated by Lebreton, Litten, Fischl and Adler, Lewis, Silvestrini, and others. Fischl and Adler claim to have produced a fatal anemia in animals by inoculation with their coccus. Kolb obtained sterile cultures of the blood in five cases, but from the viscera of three of these, three to four hours after death, he isolated a diplobacillus which caused hemorrhagic septicemia in various animals. Auche obtained both the staphylococcus and streptococcus in a fatal case, and Levi obtained in another both *streptococcus pyogenes* and the pneumococcus of Fraenkel.

The *pneumococcus lanceolatus* was isolated postmortem by Claisse and by Claude. *Bacillus pyocyaneus* was obtained postmortem from a case of *melena neonatorum* by Neumann, and *bacillus coli communis* by Dansac, Legendre, and others. Hamilton and Yates report a rapidly fatal case showing, ten hours postmortem, *bacillus aerogenes capsulatus*. McLeod observed a case closely following Malta fever, and conditions described as purpura hemorrhagica have been reported as following pertussis, tuberculosis, congenital syphilis, etc. *Negative results* are reported in a fatal case by Denys; in a purpuric complication of angina, by Legendre; and in chronic cases by Marfan, and by Millard.

In the majority of the above cases the bacterium was obtained postmortem and from the viscera, or during life, from the lesions in the skin, and was rarely demonstrated in the circulating blood. Nevertheless, there can be but little doubt that a numerous group of cases of purpura hemorrhagica exists which is referable to a variety of bacteria, as represented in the above reports.

It seems necessary, therefore, to provisionally separate the cases of purpura hemorrhagica of probable infectious origin from those which in no respect resemble an infectious disease, and are, therefore, probably acute manifestations of hemophilia.

Changes in the Blood. In most of the febrile infectious cases of *purpura hemorrhagica* the anemia is not excessive and appears to be secondary to the infection and the loss of blood. In *mild cases* the red cells are slightly diminished in number, but exhibit no other alterations. Carriere and Gilbert report cases with 3,350,000 and 3,900,000 red cells, many of which were microcytes. In more *severe cases* the red cells may be greatly diminished, in proportion to the extent of hemorrhage, but a microcytic type of red cell is usually prominent. The Hb-index is subnormal. Nucleated red cells occur when hemorrhages are large or frequent.

In a fatal case in an infant, yielding a pure culture of *streptococcus pyogenes* at postmortem, the writer found the blood to show all the characteristics of primary *pernicious anemia*. In four other cases of fatal hemorrhage in newborn infants at Sloane Maternity Hospital, the subjects had bled to death in the course of three to five days, but the blood showed only the characteristics of secondary anemia with leucocytosis. Leucocytosis has been noted by the majority of observers. In one case Carriere and Gilbert found 126,000 leucocytes, 90 per cent. polynuclear, and 5.8 per cent. eosinophile. In the writer's cases in infants the leucocytosis reached as high as 56,000, but the eosinophiles were scanty. A very marked degree of polychromatophilia was observed in two cases by Spietschka.

Hayem[1] and Bensaude have described a peculiarity in the blood of *purpura hemorrhagica* which they claim to be pathognomonic. On allowing the blood to clot in a vessel it was found that after twenty-four hours the retraction of the coagulum is very feeble and fails to express the serum, as happens in normal blood. Associated with this feeble coagulation there is a marked reduction of blood plates (200,000 to 50,000). These two features of the blood were noted in sixteen cases of *purpura hemorrhagica*, but in 152 examples of other diseases, some with purpura, the feeble clotting and loss of blood plates were never found to be combined.

Non-infectious Idiopathic Purpura Hemorrhagica.

In addition to the cases of purpura which arise in the course of infectious diseases or after various infectious processes, in which hemorrhage constitutes the chief symptom of a cryptogenic infection, there are other cases of rapidly fatal anemia attended with severe and repeated losses of blood for which a different etiology is indicated.

Some of these cases are difficult to distinguish from pernicious anemia, but differ from that disease in the absence of megaloblastic changes in the blood or marrow, the great predominance of microcytes in the blood, and the prominence of hemorrhage among the symptoms. They differ from the infectious purpura in the more persistent hemorrhage, the absence of leucocytosis, and the absence of general symptoms of an infectious disease.

Such cases have been reported by Ehrlich,[1] Engel, Muir, and others as pernicious anemia without characteristic changes in the blood or marrow. The writer can find no evidence on which to claim that pernicious anemia can exist without such changes in the marrow, and believes that these cases belong to the diseases referable to the hemorrhagic diathesis. This view is supported not only by the great dissimilarity in the blood changes of the two conditions, but by the absence of any marked deposits of iron in the viscera (Zalesky). In Muir's case the marrow of the rib consisted almost entirely of fat tissue with very few normoblasts, while the shafts of the bones were decidedly thicker than normal. Here the condition was apparently one of congenital agenesis of the marrow. The viscera contained considerable deposits of iron.

Of the various etiological factors to which fatal purpura has been attributed the non-infectious variety may be referred to any or all except the presence of bacteria. The underlying condition is probably identical with that of hemophilia of more typical course.

Changes in the Blood. In the writer's case the *clotting* appeared to be abnormally rapid, as in the course of an hour the specimen in the hematocytometer diluted 1 : 100 with 0.6 per cent. salt solution became jelly-like. Grawitz has also noticed an increased coagulability of the blood in these cases after repeated hemorrhages. The red cells are rapidly diminished in number, reaching before death an extremely low figure. In a case of the writer persistent epistaxis reduced them in three weeks to 456,000. In a case reported by Billings they numbered 483,000. In Engel's case there were over 2,000,000. Contrary to the rule in secondary anemia following hemorrhage, nucleated red cells are extremely scarce or absent. The majority of the red cells are undersized and many are of oval shape. The leucocytes are normal or reduced in number, and of those remaining a large proportion (90 per cent., Engel; 80 per cent., Ehrlich; 75 per cent., Billings) are lymphocytes. Eosinophile cells are scarce or absent.

HEMOPHILIA.

Hemophilia is an extremely hereditary constitutional anomaly which predisposes the subject to persistent and fatal hemorrhages of traumatic or spontaneous origin. The peculiar law of heredity which transmits the condition to males only through the female parents who are themselves usually exempt, as fully set forth by Grandidier and Stahel, not only marks this malady as the clearest possible illustration of the hereditary transmission of disease, but completely baffles the attempts to prove its dependence on any infectious agent.

The essential lesion has been held by many to lie in an unnatural *thinness and narrowness of the arteries* (Virchow). In somewhat altered form this theory is maintained by Immerman and Oertel, who hold that there is a *disproportion between the bulk of blood and the capacity of the blood system*, referable in part to hypoplasia of vessels (Immerman) or to hydremic plethora. Proceeding on this theory Cohen claims to have considerably lessened the tendency to hemorrhages in a pronounced bleeder by a prolonged course of diuresis and diaphoresis. In partial support of this mechanical theory are cited a few cases in which the heart was hypertrophied, and the vessels of small size, and showing fatty, hyaline, or granular degeneration.

A *local origin* is perhaps indicated by the case cited by Stengel, in which the hemorrhages were observed to occur from cuts above the neck, but never from those below.

Recklinghausen holds that a *disorder of the nervous system* is the essential cause of the hemorrhages, and Henoch believes that the local condition is one of paralytic dilatation of vessels, followed by diapedesis. The condition has been seen in some very remarkable forms in neurotic and hysterical subjects (Stengel).

Little success has followed numerous attempts to demonstrate essential **changes in the blood.** Albertoni claims to have found diminished resistance in the red cells. The remarkable therapeutic influence of calcium chloride, described by Brunton, and reported by Wallis, supports the view that the chief alteration in the blood is in the content of salts. Wright finds that the bleeding in hemophilia is diminished by administration of calcium chloride, by inhalation of CO_2, and by local application of the nucleo-albumin of the testicle, thyroid, and gastric mucosa. In morphology there is uniform agreement that the blood presents nothing peculiar. Decreased coagulability was held to be constant, by older writers (Grandidier, Lossen), and has later been observed by Schmidt and Manteuffel, who found that the blood in hemophilia requires longer than usual to clot, while its coagulation may be greatly hastened by adding a zymoplastic substance (fibrin ferment). Zavialoff claims to have found evidence that the red cells in hemophilia contain a cytoglobin, not present in normal blood, which prevents coagulation when liberated by the cells on shedding of the blood. The fibrin itself is apparently not greatly diminished, 5 per cent. having been found by Heyland; 2.6 per cent., by Gavoy and Ritter, and 4.3 per cent. by Otto (cited by Litten).

Studies in **pathological anatomy** have also failed to show any constant lesion of essential importance. In most of the viscera there are the lesions which follow acute or chronic anemia. Buhl described an unusually rich network of capillaries in the skin of one case, but the tissue was the seat of a chronic inflammatory process. Birch-Hirschfeld found normal vessels, slightly fatty heart muscle, and slight hyperplasia of the splenic stroma. Litten noted areas of granular degeneration in capillary endothelium, and irregularity in outline or widening of intercellular spaces between these cells, but suspected these changes to be artificial. In a series of cases hypoplasia of the heart furnished the basis of Virchow's theory.

The blood changes are determined by the extent of the hemorrhages, but leucocytosis has been absent in the few cases reported. In general, the blood resembles that of non-infectious *purpura hemorrhagica.*

SCURVY.

Etiology. The most important fact in the etiology of scurvy is its intimate relation to improper diet. Aside from the almost exclusive occurrence of the disease in subjects whose food has been restricted in variety, it has been repeatedly demonstrated that the malady may be promptly cured by supplying some element previously deficient in the diet. Even a change in water supply has eradicated epidemics in garrisons. Scorbutic infants rapidly recover from grave stages of the disease after such apparently trivial assistance as a change in the milk. Yet it has never been fully determined just what chemical substance is concerned in the causation and cure of the disease. It has even been claimed that diet has no relation to the malady, but all such reports have thus far, on investigation, failed to be supported, although they have demonstrated that the dietetic error may be very trivial. Thus Seeland, regarding a somewhat limited army ration as full mixed diet, came to regard the cases of scurvy under his care as of miasmatic origin. Accumulating evidence has shown that the most frequent source of the disease is a too exclusive diet of meat, especially if salted, and the absence of fresh vegetables, especially of potatoes, which are very rich in potassium.

Guided by these observations various theories of origin of scurvy have been elaborated. Among these is the belief that the symptoms were caused by excess of NaCl in the blood, a condition long since shown to characterize most forms of anemia. That the essential element in the disease consists in a deficiency of potassium in the blood was held by Garrod, Liebig, and Hirsch, but a loss of potassium from the blood, with excess in the urine, while not constant in scurvy, is observed in other conditions. Ralfe and Cantani hold that a deficiency of salts of vegetable organic acids and corresponding diminution in the alkalinity of the blood is the important factor. Wright also maintains that scurvy is a form of acid intoxication arising from a diet rich in mineral acids and poor in bases. In seven advanced cases of scurvy from the beleaguered garrison of Ladysmith he found, by his alkalimetric method, that the blood was very deficient in alkalescence $\left(\frac{N}{80}-\frac{N}{200}, \text{ normal } \frac{N}{35}\right)$. The administration of sodium lactate was very effectual in relieving the symptoms.

To those who have observed the immediate effects of correction of diet in severe cases, especially in children, this field of investigation appears most likely to offer the true explanation of the disease.

On the other hand, there are on record some very suggestive *bacteriological studies* of scurvy, pointing toward an infectious nature of the disease. The injection into animals of blood from scorbutic patients caused purpuric lesions and internal hemorrhages in the experiments of Murri,[1] Petrone, and others Babes failed to find bacteria in the blood of a series of cases and failed to produce significant lesions by injecting blood into animals, but from excised portions of the spongy gums he isolated a bacillus, regarded as identical with one previously described by Müller as common in the buccal cavity, and by injections of the comminuted tissue he produced, in two out of a series of rabbits, a form of hemorrhagic septicemia. From the visceral lesions he recovered this same bacillus along with the ordinary bacillus of hemorrhagic septicemia in rabbits. It does not appear that much significance can be attached to these results, although they are commonly quoted as strongly supporting the infectious theory of the origin of scurvy. Rosenell also recovered a somewhat similar bacillus from the viscera of a fatal case of scurvy, but inoculations into rabbits were negative. Entirely negative results were obtained by Wieruschky in a large number of cases. Most of his cultures of the blood were sterile, but in fourteen cases he found various germs to which he could not assign any relation to the disease.

In recent years the bacteriological study of scurvy has not proved an inviting or accessible field to competent bacteriologists.

A recent important study directed toward the chemistry of the blood and of digestion is that of Albertoni, who has shown that there is a marked loss or complete absence of free HCl in the gastric juice in scorbutic patients,

that gastric digestion is deficient, that intestinal putrefaction is excessive, and that there are abundant evidences in the urine of absorption of toxic products, while the absorption of fats and carbohydrates is deficient. In the blood Albertoni found a much greater reduction in iron (0.3 to 0.39 per cent.) than is seen in other anemias with equal numbers of red cells. The sodium and potassium varied between normal limits. The greenish-yellow color of the serum and the excess of pigments in the urine were evidences of active destruction of blood cells.

Changes in the Blood. The morphological changes in the blood in scurvy resemble those of secondary anemia from hemorrhage.

The red cells in cases of average severity number between 3,000,000 and 4,000,000, but with severe hemorrhages the anemia may become very grave, as in Bouchut's case, in which, after three weeks of persistent epistaxis, the red cells were reduced to 557,000. The reports of Uskow, Hayem,[2] Wieruschky, and Albertoni show that the red cells vary in number and size according to the length and severity of the disease. Litten saw many megalocytes and shrunken microcytes, evidently in severe cases. Albertoni found evidences of solution of red cells in the plasma, and an excessive number of pale cells or fragments of cells have been described by several observers. The Hb-index is low, White finding 20 per cent. Hb with 40 per cent. of red cells, while Becquerel and Albertoni found the iron-content much lower than in other anemias. Opitz and Duchek, however, reported a normal or increased proportion of iron.

The proportions of sodium and potassium salts vary as in other anemias, and the claims of Ralfe and Cantani that the alkalinity of the blood is deficient have not been supported.

On account of the frequency of inflammatory complications and hemorrhages the leucocytes are usually increased, Uskow finding as high as 47,000. Litten, however, observed no leucocytosis, and the writer, in two well-marked but uncomplicated cases, found no increase.

In Barlow's disease, which is a form of scurvy with extensive subperiosteal hemorrhages, the condition of the blood varies greatly, according to the general condition of the patient and the number and extent of the hemorrhages. All grades of post-hemorrhagic anemia have been observed, up to Reinert's fatal case, in which the Hb fell to 17 per cent. and the red cells to 976,000. The disease is often associated with rachitis.

HEMOCYTOLYSIS.

When the isotonic tension of the plsma falls below that of the red cells, the Hb may become dissolved in the circulating blood, and the shadow-like remnants of the stroma of the cells are distributed in the plasma. In other conditions, even without lowered isotonic tension of the plasma, the red cells may be split up into fragments. The purest examples of the former variety of destruction of red cells are perhaps seen after extensive infusions of salt solution, while the latter variety is observed especially from the ef [illegible] ertain blood poisons—*e. g.*, potassium chlorate. Usually the two processes are combined, as in the anemias and infectious diseases, while in some conditions the exact manner of the solution of Hb remains unexplained.

The chemical processes in the cell leading to the solution of Hb are somewhat obscure, since this constituent is probably held in chemical combination with the stroma and not merely held *in situ* by a hypothetical cell membrane. Thus the most extreme form of hemoglobinemia (paroxysmal) may occur in blood of comparatively high isotonic tension, while the high grades of globulicidal activity of the plasma in infectious diseases are not always associated with hydremia. Hemocytolysis is followed by diminution in the alkalescence of the blood owing to the discharge of phosphoric and glycerophosphoric acids into the plasma during the passage of Hb into solution (Kobert).

The studies that have followed the work of Bordet and Ehrlich on hemolysis have elucidated the conditions governing this obscure process while leaving its true nature unexplained. It has been shown that the serum of one animal possesses, as a rule, power to dissolve the red cells of heterologous species. This result depends upon the interaction of two agents, one of which, the "immune body" (amboceptor), is specific for each species of animal; while the other, the complement (alexin), may be furnished by the animal whose blood cells are dissolved. The immune body resists heating to 55° C., which usually destroys the alexin.

When washed blood cells are treated with hemolytic serum heated to 55° C. (alexin destroyed), hemolysis fails until fresh unheated serum is added. Different immune bodies are developed in the process of immunization against different heterologous bloods, and Ehrlich claims that there is a similar multiplicity of alexins. In any event he believes that the interaction of these various agents is of the nature of a chemical union, each of the bodies being consumed in the process, while the subsequent changes in the cell are of a digestive nature, as by ferments. This view has been actively combated.

Buchner and Gruber believe that the alexin unites directly with the red cells without the intervention of an amboceptor. Baumgarten argues that hemolysis always results principally from the changes in isotonic tension. In hemolytic serum the same changes in the red cells occur as in hypisotonic salt solution, viz , shrinkage and discharge of Hb in droplets. Baumgarten finds also that when foreign red cells in 4 per cent. salt solution are added to inactivated specific sera (alexin destroyed by heat) hemolysis begins at once. Here the alexin appears to be nothing more than salt. He also claims that abrin and ricin dissolve red cells only in hypisotonic solutions.

The morphological changes occurring in dissolving red cells are also not fully identified, although it appears more and more probable that many forms of degeneration previously described in red cells are the precursors of the complete solution of Hb. The polychromatic degeneration of Maragliano, that of Gabritschewsky, and the granular degeneration of Grawitz are probably morphological signs indicating approaching or progressive solution of the cell. Fukuhara has described the morphological changes in blood cells treated with hemolytic sera, hypisotonic salt solution, and 2 per cent. soda solution. The changes induced were in each case similar, except that the sera agglutinated the cells. The cells became swollen and dissolved with slight tendency to disintegrate, or they became

PLATE XIII.

Fig. 1.

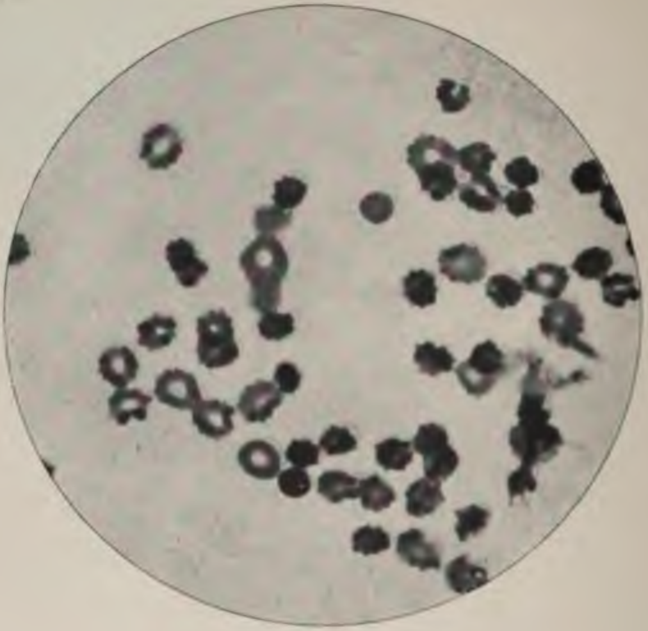
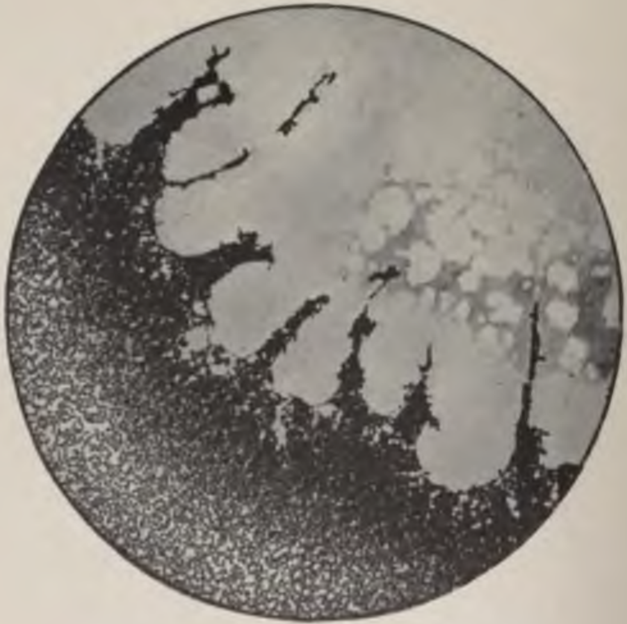


Fig. 2.



Agglutinated Red Cells.

Fig. 1. Stained agglutinated human red cells from a specimen prepared in the test tube.
Fig. 2. Effect of a drop of hemolytic serum added to a dried smear of human red cells.
Photographed by Dr. B. H. Buxton.

crenated and the protoplasm broke up into granules or discharged blood plates, which subsequently dissolved (Plate XIII.).

From extensive study of the anemia following pyrodin poisoning Tallquist concludes that *the presence of microcytes is a sign of hematolysis, while megalocytes indicate regeneration of the blood.* The dissolved Hb imparts to the plasma, in the eosin-stained specimen, an abnormal reddish tinge, and discolors the serum. When present in moderate amount it may be gradually transformed by the liver into bile-pigment, which is occasionally reabsorbed from the biliary passages, causing hematogenous jaundice. The presence of an excessive amount of Hb in the plasma is apparently the chief reason why it is sometimes excreted by the kidneys and appears in the urine as hemoglobin or methemoglobin. During its passage through the liver, kidney, and other tissues a considerable portion of the iron is retained in the granular or diffuse form of hemosiderin.

Under some conditions not fully understood, but believed by Thoma to consist essentially in the relative absence of oxygen, disintegrating red cells are transformed largely into brownish granules or crystals of hematoidin. This change usually takes place without the intervention of cellular activity (Thoma). It frequently occurs postmortem.

Broken fragments of red cells and the stroma of disintegrated corpuscles float in the plasma for a time and may be observed in the stained specimen, but are gradually englobed by phagocytic cells and lodged in various organs, principally in the spleen.

Hemolysis of Infectious and Toxic Diseases. The anemia of the infectious diseases, especially in grave septic conditions, is established rapidly and to a large extent through the direct solution of red cells by the globulicidal plasma. The condition is often attended during life by hemoglobinuria, and after death extensive deposits of blood pigment are found in all viscera, especially in the spleen and liver.

The degree and character of the hemolysis of some infectious diseases have been studied in detail by many observers, and the active principles of many bacteria have been isolated and tested, as tetanolysin (Madsen); pyocyanolysin (Bulloch, Lubenau); staphylolysin (Neisser, Wechsberg); typholysin (Levy).

Isolysis. Several observers, including Ascoli, Kraus and Clairmont, and Halpern have studied the agglutinating and solvent action on human red cells of the sera of human subjects. This action of so-called normal human blood upon red cells of other human subjects is spoken of as *isolysis* and the active agents *isolysins.* Ascoli found in the serum of seventeen healthy subjects varying degrees of agglutination and solution of the red cells of other subjects, but not in dilutions greater than 1 : 20. Kraus and Clairmont found one active serum in ten healthy subjects, and Donath four in twelve subjects.

All observers agree that the isolysins are probably developed from the absorption of disintegrating red cells or from the absorption of bacterial products. Ascoli found the red cells of anemic subjects very susceptible to many normal sera, but secured no effects from the sera of several cases each of chlorosis, abscess, acute rheumatism, pleurisy, plumbism, nephritis, anchylostomiasis. Actively hemolytic sera were obtained from cases of pneumonia, tuberculosis, cancer, malaria, and typhoid fever, but not in every case.

Kraus and Clairmont, in 150 cases, found many active sera, especially in scarlatina, typhoid fever, pneumonia, and tuberculosis. The effects of such sera are, of course, referable to bacterial products and disordered metabolism, but there appears to be only a difference of degree between their action and that of the isolysins in apparently healthy subjects.

The presence of agents dissolving leucocytes, "leucocidins," has been demonstrated in the blood in some infectious diseases, and they may be developed by injections of these cells from one animal into another. Van de Velde, and Neisser and Wechsberg find that the leucocidins develop strongly in cultures of *staphylococcus pyogenes aureus* on the fourth day. That they are not identical substances with the hemolysins is indicated by the facts that they appear in the cultures at different times, while the addition of living leucocytes to the cultures neutralizes the leucocidin, but has little or no effect upon the hemolysin.

In malaria various forms of destruction of red cells occur, which will be considered later.

The possible therapeutic use of hemolytic serum has been studied by Cantacuzene. He found that 15 c.c. of a hemolytic serum killed rabbits in one to two minutes, with spasms and cyanosis. Much smaller doses diminished the red cells in forty-eight hours to 300,000, Hb 35 per cent., and gave marked leucocytosis. One-tenth to one-thirtieth c.c. increased the red cells to 8,800,000, but after eight days they fell to normal. Repeated minimal doses raised the red cells to 9,000,000, Hb 110 per cent , and caused leucocytosis.

Bielonovsky reports equally good results in man.

Special Conditions in which Blood Cells are Rapidly Destroyed.

Burns. The study of the pathology of the blood in burns appears to have begun with the observations of Baraduc, 1862, who attributed the numerous thromboses to thickening of the blood from exudation and venous stasis. In 1865 Schultze noted the crenation and minute subdivision of red cells exposed to 52° C. This process can be readily followed in a fresh specimen heated over a flame.

The experimental study of the tissue changes in burns, thus began, led Wertheim, v. Lesser, Silberman, Welti, Demme, Pawlowsky, Tappeiner, Markusfeld and Steinhaus, and others to conclude that death from burns is referable to destruction of blood cells with thromboses, and to concentration of the blood from exudation and stasis, while some of the authors lay much stress on the toxic effects observed in the kidneys or other viscera.

The changes observed in the red cells were extreme in degree, but not always extensive. The cells were distorted, subdivided, broken up into globules or granules, many "shadows" remained, the Hb often gathered in circumscribed masses in the cell, and basic staining granules or areas appeared in the cells.

On the other hand, Hoppe-Seyler and many later observers could not find sufficient changes in the red cells to account for death, and they favor the belief that death is due to a toxic substance developed in the blood and tissues. Fraenkel, from a study of seven cases at autopsy, concluded that death could be explained only as the result of toxemia. Schjerning and others thought the poison must be the potassium salts set free from the red cells. Kajanitzin isolated from the blood of burned animals a poisonous ptomain belonging to the group of cadaveric muscarins. Reiss extracted from the urine poisonous bodies resembling pyridin.

The results of neither of these studies, however, have enjoyed full verification, and Pawlowsky rejected the toxic theory after finding that aseptic treatment of the blood and viscera of burned animals left them non-toxic.

Bardeen, from the histological study of five cases, concludes that toxemia must exist to account for the degenerations of the tissues, and especially the focal necroses in the lymph nodes and liver.

Hemoglobinemia and hemoglobinuria have been described by Hoppe-Seyler, Tappeiner, and others. Locke has contributed the only detailed study of the numerical changes in the blood cells. He found that an immediate increase of 1,000,000 to 2,000,000 red cells occurs in severe cases, and 2,000,000 to 4,000,000 in fatal cases, while the blood is thick and dark purple in color. A rapidly rising leucocytosis occurs in all cases, reaching 30,000 to 40,000 in recovering cases, and usually more than 50,000 in fatal ones.

The percentage of polynuclear cells is not greatly increased (60 to 87 per cent.). He found slight morphological changes in the red cells.

Snake Poison. The fatal effects of the bites of many snakes is largely referable to very active agglutination and solution of red blood cells by the proteid constituents of the venom, as demonstrated by Mitchell. A serum capable of inhibiting the action of the poison has been prepared by Sewall, Calmette, and Frazer. It has recently been shown by Flexner and Noguchi that snake venom is inert upon washed blood cells, and hence that it contains no complement, but only amboceptor, and that it destroys bactericidal properties of blood serum through action on the complement of the serum.

Auche and Vaillant observed rapid solution of the red cells in proportion to the quantity of snake poison injected by bite or syringe. They found reductions of 1,500,000 to 2,000,000 cells in short periods. In surviving animals many nucleated red cells appeared. Polynuclear leucocytosis developed in severe cases, both fatal and favorable, in one-half to two hours, lasting three to four days or until death.

Arsenic. Vryens finds that hemoglobinemia occurs rarely in acute poisoning by arsenious acid and no destruction of red cells follows doses less than 0.01 gm. per kilo of body weight (9.6 gm. for 140 pounds). Stockman and Greig found no changes in the red cells from toxic or fatal doses in rabbits. Muir observed thirty-one cases of chronic arsenical poisoning in which the red cells averaged 4,947,000, Hb 92 per cent. The marrow showed distinct increase of normoblasts. Mann and Clegg report 2,700,000 and 1,800,000 red cells in chronic cases, and they say that anemia may be very severe in cases which recover.

Fumes of arseniuretted hydrogen are very active, when inhaled, in dissolving red cells, producing hemoglobinemia, hemoglobinuria, with Hb-infarcts in the kidneys, and acute degeneration of the viscera.

Poisoning by toadstools, by the alkaloids of *quillaia saponaria* (saponin, sapotoxin), of *solanum*, and other motor depressants, dissolves red cells in the blood and leads to hemoglobinuria (Kobert).

In a fatal case of **poisoning by guaiacol,** Wyss observed rapid destruction of red cells, finding many degenerated, fragmented, shrunken red cells and megalocytes on the second to third days. The white cells were considerably increased and the majority of these were lymphocytes.

Transfusion of Salt Solution, Serum, and Defibrinated Blood. Siegel and Schram both found no improvement in the regeneration of the blood from the transfusion of salt solution or of serum in animals after bleeding, and while it has since been shown that the regeneration is somewhat more rapid and complete after salt infusion, yet this procedure must be regarded as of more value as a means of saving life than as a stimulant to blood formation. The direct effects on the blood of infusion of defibrinated blood seem to be much more favorable. On the other hand, after the transfusion of blood, the above observers, and others, have found a rapid increase in the number of cells, and Bizzozero reported the same effect after transfusion of defibrinated blood in animals. Quincke was one of the first to note an increase in red cells in pernicious anemia as a result of transfusion of blood, and similar observations have been made by Ziemann in anemia and scurvy. It would seem, from the observations of Bizzozero and of Bareggi that the red cells are quite resistant to the process of defibrination and injection. Of the immediate effects of salt infusion upon the blood of the human subject there are a few reports at hand which indicate that it has considerable influence in lowering the number of red cells and increasing the leucocytes.

Methemoglobinemia. Various poisons not only dissolve red cells, but at once transform the hemoglobin into methemoglobin. The blood in such cases may exhibit a distinct chocolate color.

Potassium chlorate is one of the most frequent forms of poisoning which cause destruction of red cells with methemoglobinemia. According to v. Mering the $KClO_3$ acts directly upon the cells and is itself thereby reduced, while Marchand, Falek, and others hold that the serum is first altered. Brandenburg reports a fatal case in which the red cells fell in six days from 4,300,000 to 1,600,000. Many of them were deformed. The blood and serum were chocolate-colored and gave, during the first five days only, the spectrum of methemoglobin. There was marked leucocytosis (20,000).

In a case fatal in six days Jacob noted progressive pallor of the red cells, numerous rouleaux of "shadows," little or no red-cell detritus, and numerous poikilocytes and megalocytes. The cells increased from 1,800,000 to 2,200,000 before death.

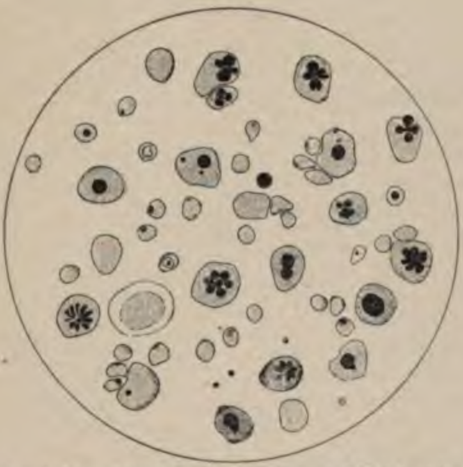
There was continuous leucocytosis, reaching 80,000, while the nuclei of the white cells were very pale and the neutrophile cells were deficient in granules. In a case which recovered Markwedel observed all the above changes, and while the leucocytosis subsided on the fourth day some red-cell shadows persisted for two weeks.

Sticker reports a severe case of poisoning after a vaginal douche by solution of **chromic acid**, the patient passing brownish urine. He refers to other fatal cases with similar signs.

Nitrites. Poisoning by nitroglycerin and by amyl nitrite is said to occur in the manufacture of these chemicals, and to be followed by methemoglobinemia.

Nitrobenzol poisoning was studied in a fatal case by K. Ehrlich and Lindenthal.

FIG. 36.



Acute degeneration of red cells. Nitrobenzol poisoning. Subdivision of cells and appearance of basophilic granules in them. (K. Ehrlich, Lindenthal.)

Ten hours after the initial symptoms the blood was chocolate-colored, the serum brownish, and spectral analysis showed the presence of methemoglobin, which disappeared by the eighth day. The red cells were rapidly reduced, 2,275,000 on the fifth day, and falling to 900,000 before death on the nineteenth day. Poikilocytosis appeared on the third day and soon reached a remarkable degree. Polychromatic and fragmented cells were abundant. Nucleated red cells were first seen on the third day, and thereafter in very large numbers and of all sizes. On the ninth day the leucocytes, previously low, rose suddenly to 61,000, and the nucleated red cells were reported at 24,700. Judging from the authors' plates it was, perhaps, difficult to distinguish between karyorrhexis in some nucleated cells and extreme forms of the granular degeneration of Grawitz. The Hb fell steadily to 40 per cent., which, with 900,000 cells, was a remarkably high Hb-index. There were many myelocytes among the white cells, so that at one time the blood presented the appearance of leukemia. The bone-marrow was not examined. These interesting observations recall the efforts of Bignami and

Dionisi to produce experimental pernicious anemia by means of toluendiamin. The morphological characters of the blood described by Ehrlich and Lindenthal probably represent an extreme degree of the effects upon the blood of the entire group of anilin poisons.

Mohr observed six cases of *benzol* poisoning among workers in benzol factories. In all there was methemoglobinemia, schistocytosis, and hemoglobinemic degeneration, while nucleated red cells, microcytes, and macrocytes were numerous.

Pyrogallol and **pyrogallic acid** have caused death with symptoms of methemoglobinemia (Grawitz).

Antifebrin has been found to produce extreme methemoglobinemia in animals (Lepine) and in man (Müller), but neither of these observers could find any morphological changes in the blood of subjects dying from this cause.

Brown describes a case fatal in eight days after taking sixty grains of acetanilid. There was progressive anemia from destruction of blood, the red cells falling to 1,100,000, Hb 30 per cent., while of 25,000 nucleated red cells 20 to 40 per cent. were megaloblasts. The leucocytes rose to 66,450 with proportions normal. The alkalinity was said to be greatly reduced and there was hematoporphyrinuria.

Antipyrin produces toxic symptoms similar to those of antifebrin, but reports on the examination of the blood in severe cases are wanting (see Falk, Müller).

Toluendiamin poisoning was studied experimentally by Schwalbe and Solley, who found that it produced granular degeneration of the red cells with extrusion of blood plates.

Poisoning by CO. A bright cherry-red color of the blood is a characteristic sign in cases of "gas poisoning," and is referable to the presence of CO-Hb. The methods of diagnosis of this condition by spectral analysis have already been considered.

Poisoning by Hydrocyanic Acid. The bright red color of the blood in this condition is referable to the presence of cyan-methemoglobin (Kobert), which gives a characteristic spectrum. The manner of death is believed by Geppert to be through inhibition of the metabolic processes in tissues, so that CO_2 is not discharged and oxygen not absorbed. Morphological changes in the blood have not been reported.

Paroxysmal Hemoglobinuria. This remarkable condition, consisting essentially in the rapid solution in the blood stream of enormous numbers of red cells, followed by the appearance of Hb in the urine, was recognized by Stewart more than a century ago (1794), and has been widely studied in its various phases ever since. Chvostek's monograph reviews the literature up to 1894.

Etiology. The most peculiar feature of this malady is the apparently trivial nature of the exciting causes of solution of the red cells. Slight exposure to cold, even of a part of the body, has been the most common exciting cause, many confirmed subjects being able to induce an attack at will. Other exciting conditions have consisted in mental excitement or nervous irritation, and muscular or nervous exhaustion.

In recently malarious subjects Tomasselli and Koch have placed beyond doubt that quinine is a frequent exciting agent. An infectious origin has been suggested by Layral and by Cima. The underlying conditions which predispose to the disease are known to include only constitutional syphilis (Boas, Gotze) and malaria (Legg), but in a considerable proportion of cases neither of these factors appears to exist.

Pathogenesis. That a vasomotor neurosis is prominently concerned in the solution of blood cells is strongly suggested by the frequency with which nervous influences have been known to precipitate the attack. This theory, first suggested by Dapper in 1868, has been uniformly maintained up to the present time by the majority of observers. The further attempt to explain the destruction of blood by this means has as yet been unsuccessful. Chvostek concluded that as a result of malaria, syphilis, etc., the red cells possess diminished resistance, not to cold, but to mechanical traumatism, and that when the circulation is disturbed by vasomotor contraction of peripheral vessels the fragile red cells become dissolved. He supported his view by showing, as Dapper had done, that the disturbance of circulation following ligation of a finger may cause solution of red cells in the absence of cold. With Pavy, McKenzie, Rosenbach, and others, he attributed to the kidney a special importance in the process. Murri[2] has offered evidence to show that the red cells are abnormally susceptible to the effects of cold, and that by paralytic dilatation of peripheral vessels they become exposed to cold and dissolved. But Rodet failed to find by experiment any lack of resistance of the red cells against cold, and numerous cases have been observed to follow muscular and mental overexertion (Fleischer, Strobing). Ehrlich supposes that in the predisposed subjects exposure to cold causes the development of "ferments" which dissolve the red cells.

Postmalarial Hemoglobinemia. The relation of *blackwater fever* to malaria has been the subject of much discussion, the opposing views in which are fully presented in the studies of Koch and of Plehn. From the observations, correlated and largely contributed by these observers, it is evident that hemoglobinuria arises:

1. As a direct result of the action of the malarial parasite upon the blood.

2. As an immediate effect of the administration of quinine on patients predisposed to hemoglobinemia from present or pre-existing malarial infection.

3. It is probable that hemoglobinuria results from simple exposure to cold, or to other exciting agents, in subjects who have acquired the predisposition through previous malarial infection.

Plehn fully demonstrates that blackwater fever may occur in patients suffering from malaria, without the administration of quinine, and with or without notable exposure to any of the ordinary exciting causes of the condition. Numerous cases collected or observed by Plehn leave this fact apparently beyond doubt.

Plehn's report that a special variety of parasite is concerned in blackwater fever has not been confirmed, and it is probable that the author mistook the peculiar appearance of granular degeneration of red cells for minute parasites. The most extensive changes of this type observed by the writer were seen in severe malarial cachexia at Montauk, 1898, and in many instances the altered red cells presented a remarkable resemblance to corpuscles infected with young malarial parasites.

On the other hand, Koch has recently drawn contrary conclusions from a thorough study of forty-one cases. He finds that hemoglobinuria does not necessarily follow even when 80 per cent. of the red cells are infected with parasites. The great majority of his cases developed a few hours after the administration of quinine. With regard to the relation of malaria to blackwater fever he found that: (1) Parasites may be present in the blood, but there is no relation between their numbers and the severity of the hemoglobinuria. (2) The patient may have suffered from malaria some weeks or months previously. (3) In two fatal cases in the tropics he found neither parasites in the blood nor pigment in the viscera.

Koch concludes that life in the tropics, and especially malarial infection, predispose certain individuals to hemoglobinuria, but that the attack is invariably excited by quinine, or occasionally by exposure to cold or heat, etc. His further claim that no cases are referable to malaria alone cannot be accepted in the face of the observations of Plehn and many others, of fatalities occurring in the course of malarial infections which have not been treated by quinine.

Changes in the Blood. The destruction of RED CELLS very promptly reduces their numbers in the peripheral circulation, as shown by the reports of numerous observers. Bristowe and Copeman in a series of attacks induced in the same patient found a maximum loss of red cells of 129,000 to 824,000. In an idiopathic case of moderate severity Grawitz found a loss of 1,130,000 following a single paroxysm. Usually the reduction in cells is not so marked, and Grawitz has explained this fact by showing that, from vasomotor spasm and increased diuresis during the attack, the blood is considerably concentrated, since the dry residue of the blood and serum is considerably increased after the seizure, notwithstanding the loss of cells. For the same reason and because the Hb is not immediately removed from the serum, the percentage of Hb is not excessively low during or immediately after the paroxysm. Indeed, Frazer found an increase of 10 per cent. of Hb about an hour after the beginning of an attack. Usually there is a loss of 5 to 10 per cent. Ponfick claimed that at least one-sixtieth part of the Hb of the blood must be lost before it will appear in the urine.

The appearance of the blood in the stained specimen taken shortly after the paroxysm usually gives evidence of active destruction of blood. The red cells may fail to form rouleaux, although having possessed this property just before the attack. Many very pale or shrunken or fragmented cells have been observed in some cases, while in others the red cells were found to contain a normal amount of Hb. After repeated attacks megalocytes appear. On the other hand, Kohler and Obermayer found no morphological changes in a case losing 650,000 red cells and 10 per cent. Hb. The presence of polychromatic cells or of granular degeneration of red cells has apparently not been reported, but in a case of blackwater fever, shortly before death, the writer found the latter form of degeneration in extreme degree. Frank noted in a paroxysmal case that the red cells in the fresh condition looked pale and brownish. The serum has been found distinctly tinged by the dissolved Hb. Bristowe and Copeman found pigment granules, crystals of Hb, and colorless crystals resembling Charcot's.

The LEUCOCYTES were found by Frazer moderately increased (21,000), but the majority of observers have reported them as normal, or, if above normal, as being little affected by the paroxysm. The blood plates have been reported as very much increased (Frazer, Mesnet), as seems very probable from the extensive destruction of red cells.

The COAGULABILITY OF THE BLOOD is normal or increased. Hayem[3] noted that the rapidly formed clot softens very soon, which Chvostek finds is observed in other conditions. The resistance of the red cells, as determined by Hamburger's method, was found by v. Hoff to have fallen to 38 per cent. NaCl, and Sabrazes, using Malassez's method, also found it reduced.

Following the destruction of red cells there is usually a reduction in the ALKALINITY OF THE BLOOD. Kobert believes this to be due to setting free of phosphoric and glycerin-phosphoric acids during the separation of oxyhemoglobin from the red cells.

The regeneration of the blood is, in uncomplicated cases, very prompt, Bristowe and Copeman finding an increase of 500,000 cells after five days, and 600,000 after six days. Most confirmed cases, however, suffer from a moderate or marked grade of chronic anemia, sometimes associated with enlargement of the spleen.

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CHAPTER XVIII.

MISCELLANEOUS CONSTITUTIONAL DISEASES.

DIABETES MELLITUS.

THE results of older observers led to conflicting opinions regarding the condition of the blood in diabetes, since it appeared that in cases of about equal severity the Hb, red cells, and albumins might in one patient be increased and in another diminished, or might vary considerably at different times in the same patient, not infrequently rising above normal.

The studies of Henocque were among the first showing that while individual differences may be considerable, yet in a series of cases the Hb does not vary greatly from the normal. Leichtenstern, finding an excess of Hb in an advanced case and a diminution in an early case, was led to refer the anomaly to the effects of diuresis in concentrating the blood. Although the extent of the diuresis depends largely upon the amount of sugar secreted, a very close relation between the Hb of the blood and the sugar of the urine has not apparently been established. Yet the above logical explanation has been generally accepted, and the marked changes observed in the blood of many diabetics may be referred to the uncertain balance between the amount of water absorbed and that excreted in the urine.

In the late stages of the disease other factors enter to further disturb the condition of the blood. General failure of nutrition belongs to the advanced disease and the blood must suffer proportionately. Yet even in extreme cases the anemia is commonly masked by the concentration of the blood through diuresis, and it becomes necessary to class the blood of advanced diabetes, with excess of Hb and cells, as among the examples of oligemia or oligoplasmia.

From these considerations it is evident that the estimation of Hb and the counting of red cells are of little use in diabetes unless very carefully controlled by reference to the diet and urinary excretion. Thus, the writer found in an emaciated anemic case, with advanced phthisis, 78 per cent. of Hb and 4,100,000 red cells. In most reported cases in which the daily urine measured over three litres the red cells approached the normal figure.

In Grawitz's case of *coma* the red cells rose from 4,900,000 three weeks previously to 6,400,000 five hours after the onset of the coma, and the dry residue of the blood from 21.4 per cent. to 24.75 per cent. Possibly the result was in part referable to cyanosis. Habershon reports three cases of coma with excess of red cells (maximum, 6,640,000). They all suffered from dyspnea, but in one the cells were lower than they were a week before death. In a cyanotic comatose case examined by the writer there were 6,800,000 red cells.

The general character of the blood in diabetes is well illustrated in the cases reported by James, as follows :

No.	Red cells.	Hb.	Specific gravity. Roy's method.
1	6.73	66	1.056
2	6.10	61	1.059
3	4.80	58	
4	5.25	60	
5	5.60	65	
6	3.55	52	1.054
7	......	52	1.060
8	5.30	75	1.056
9	6.28	108	1.055
10	5.38	96	1.055
11	5.64	112	1.056
12	6.20	112	1.057
13	4.46	55	1.054
14	6.00	96	

James concluded from these results that the excess of red cells in diabetes cannot result from concentration of the blood, otherwise the specific gravity would have been higher. Yet, considering the percentage of the Hb, the gravity in the above cases is distinctly above the usual figure, indicating relative anhydremia with marked reduction of Hb, but with slight loss of red cells.

Leucocytes. The leucocytes, in the average hospital case if uncomplicated, are normal, subnormal, or moderately increased. In seven of twenty untreated cases Habershon found less than 10,000 leucocytes, in the others a leucocytosis reaching a maximum of 19,800, and being rather uniformly present in the severer cases. With the advent of severe symptoms he found the leucocytes to increase, and in three cases of coma there was pronounced leucocytosis (maximum 28,500). Cabot found no leucocytosis in thirteen cases, and only 4200 in a comatose patient. The writer observed leucocytosis in four patients, one comatose, but autopsies revealed phthisis, suppuration, or pneumonia.

Chemistry. The chief chemical alteration demonstrated in the blood is a pronounced increase in sugar. While normal blood contains 0.05 to 0.15 per cent. of sugar, Pavy and Seegen have found as much as 0.6 per cent. in severe diabetes, and Frerichs found the sugar of the blood to vary between 0.38 to 0.44 per cent., while that of the urine amounted to 5.5 to 8.4 per cent. Naunyn obtained 0.7 per cent. in the blood of a fatal case in which the urine contained 4 per cent. In Klemperer's[3] "renal diabetes" the sugar of the blood remains normal.

Henriques and Kolish have lately claimed that an excess of preformed sugar exists in the blood *only in alimentary glycosuria*, while in diabetes the preformed sugar of the blood is but slightly above normal. They find, however, that in diabetes the blood contains a marked excess of *jecorin* in combination with albumins which, during excretion, is split up into sugar and lecithin.

Lepine and Barral believe that diab [illegible] lts from the *failure* *of a glycolytic ferment* elaborated by the pancreas and normally present in the blood, but this theory has not been demonstrated (see Glycolytic Ferment).

Fat, occurring in traces in normal blood, is usually increased in diabetes and may become extremely abundant. Usually microscopic examination is required to demonstrate the minute extracellular globules in specimens stained by osmic acid, but often the fat rises to the surface in demonstrable quantity if the specimen is centrifuged in the hematokrit, and occasionally the blood is milky from the abundance of fat. Its origin in the blood is not understood, but Van Noorden says that it is not connected with a previous ingestion of fat, as is the case with some other forms of lipemia. In one case Aschoff separated 3.44 per cent. of fat from the blood.

Glycogen in the blood of diabetes has been demonstrated in marked cases by Gabritschewsky, who found it both in plasma and leucocytes, but the significance of the brownish, extracellular granules has been brought into question. Livierato could find but little glycogen in the plasma and none in the leucocytes. Futterer claims to have demonstrated in the brain and medulla many thrombi composed of glycogen. Of five cases of diabetes the writer found in each a few leucocytes with many glycogen granules, while in the plasma were brownish-staining granules of which the significance seemed uncertain. The glycogen-holding leucocytes were less abundant than in cases of pneumonia.

Alkalinity. The alkalinity of the blood in normal subjects varies between 300 and 400 mg. of NaOH per 100 gm. of blood, and is always diminished in patients who are much reduced in strength. In diabetes, when the patients are suffering little general disturbance, the alkalinity of the blood has been found to be very slightly reduced (Minkowsky, Kraus, Lépine). When diabetes reaches the stage of excretion of oxybutyric acid in the urine, the alkalinity of the blood has been found to be lower than in any other known condition, falling as low as 40 mg. of NaOH per 100 of blood (Van Noorden). In diabetic coma Minkowsky found as low as 3.3 vol. of CO_2 per 100 of blood. It is especially in diabetes that a distinction between alkalinity and the alkali-tension referable to diffusible alkali appears to be of importance, as chiefly in diabetic coma did Brandenburg find a marked reduction in the alkali-tension of the blood.

This relative acidemia has been referred to the presence in the blood of various acid products of proteid metabolism, β-oxybutyric and other fatty acids. These acid products are supposed to act either as direct poisons, or indirectly, by lowering the alkalinity of the blood. The basis of this theory is found partly in the demonstration of diminished alkalinity in the blood, but largely in the abundance of these acids or their salts in the urine.

OBESITY.

The majority of healthy obese subjects show an excess of Hb, as is clearly indicated by the extensive observations of Kisch, who found a notable excess and maximum of 120 per cent. of Hb in 79 of 100 fat subjects, while in the other 21 it was diminished, reaching 55 and 60 per cent. in some instances. The existence of distinct *anemia* in some apparently healthy fat subjects had long been accepted, but the opinion was rendered much more certain by Leichtenstern's observation of four such cases, showing considerable reduction of Hb. Oertel also found an excess of 5 to 8 per cent. of Hb, and Grawitz reported an increase of the dry residue of the blood in very fat subjects. It appears, therefore, that there is a distinct tendency toward true plethora associated with obesity, while the anemia occasionally observed, as in the majority of Kisch's anemic cases, is referable to other causes.

Grawitz, in two cases, observed a considerable reduction in dry residue both of the whole blood and of the serum, as a result of a course of treatment which reduced the body weight ten to twelve pounds.

That the plethora of obesity is sometimes more apparent than real is suggested by the constant perspiration, frequent dyspnea, and tendency toward venous congestion, from which these subjects suffer.

ADDISON'S DISEASE.

Established cases of this disease are usually attended with a severe grade of anemia. In four cases reported by Tschirkoff the red cells before treatment numbered from 2,733,000 to 3,280,000. In three of Cabot's cases, and in four examined by the writer, the same grade of oligocythemia was observed, while Neumann reported more severe anemia with 1,120,000 red cells in one case. Morphological changes in the red cells are not marked, but microcytes are sometimes quite abundant.

Leucocytes are commonly diminished, sometimes slightly increased, and are subject to the usual variations of secondary anemia. Eosinophile cells were present in high normal proportions in Cabot's and in the writer's cases, while Cabot found some eosinophile myelocytes in one case.

Neusser claims that the relative lymphocytosis is an unfavorable sign in Addison's disease, and that it commonly increases as the patient gets worse. Hamel doubts this statement, as in a case under his care there were once 52 per cent. of lymphocytes, declining to 38 per cent. when the patient died. Hamel believes that oligemia is an essential feature of the disease, and accounts for the anemic appearance of patients whose blood is of normal quality. He and Neusser regard distinct changes in the quality of the blood as indications of some lesion of the adrenals or viscera other than tuberculosis.

Opposed to the typical cases are others reported in which the red cells were in excess of 5,000,000, reaching, in Neumann's observation, the remarkable figures of 7,700,000, while Tschirkoff found that the Hb in early cases might exceed the normal. Lucatello found the iron content of the blood higher than the Hb would lead one to expect. It appears likely that disturbances in the circulation may be responsible for some of these anomalous results, but the subject requires further observation.

The deposits of pigment in Addison's disease have been referred by Riehl and Afanissiew to the results of minute thrombi and hemorrhages in the superficial vessels, and Afanissiew observed the disintegration of red cells in local pigmented areas. V. Kahlden, however, showed these lesions to be inconstant and probably of secondary significance.

An interesting contribution to the pathology of Addison's disease, bearing on this point, has been furnished by Tschirkoff, who examined the blood of two cases with special reference to the pigments, by means of Glan's spectrophotometric method, by which he was able to estimate the relative amounts of oxy-Hb and reduced Hb. By this method he found in advanced cases a much greater proportion of reduced Hb than is present in normal blood, and at times more reduced Hb than oxy-Hb. During improvement he found an increase in the proportion of oxy-Hb at the expense of the reduced Hb, but without corresponding increase in the total Hb of the blood. He found evidence pointing to the presence of methemoglobin in the blood, but could not observe any quantitative relation between these changes in the Hb of the blood and the pigmentation of the skin. He concluded that Addison's disease is associated with a qualitative rather than quantitative change in the hemoglobin of the blood, with a corresponding failure to furnish oxygen to the tissues. His results have not yet been verified.

GOUT.

From the comparatively few recorded observations it is evident that acute gout has little effect upon the **red cells** and **Hb** of the blood, in which respect it differs from acute rheumatism. In chronic gout, also, Duckworth found cases with inappreciable anemia and concluded that when the disease is associated with anemia the condition of the blood is referable principally to complications such as hemorrhage, nephritis, lead poisoning, bad hygiene, etc. The writer found uniform but moderate anemia in a series of chronic cases examined at Roosevelt Hospital, the patients all coming from the poorer classes.

Leucocytes. The leucocytes are increased in acute attacks. Watson observed marked leucocytosis in an acute attack with a considerable percentage of large mononuclear vacuolated cells without neutrophile granules, which he regarded as myelocytes. Da Costa also observed slight leucocytosis, 14,000, and a few myelocytes. In the chronic cases leucocytosis of moderate grade may be observed, but it is difficult to determine its relation to the gouty process, as many of these patients suffer from other complaints. Neusser[2] mentions the usual presence of mixed leucocytosis in a series of 100 cases of the uric acid diathesis.

Chemistry. The chemistry of the blood in gout has for many years been the subject of extensive investigation, and while the presence of abnormal principles has been demonstrated an important relation of these principles to the pathogenesis of the disease has not been established.

Garrod first demonstrated an excess of uric acid in the shed blood, finding 0.025 to 175 grains of uric acid for 1000 grains of serum, in five cases during and shortly after the attack, and this uricacidemia has been noted by Salomon,[1] v. Jaksch, and Klemperer.[1] Recently Magnus-Levy[1] failed to find, in several cases, any constant variations in the amount of uric acid which might be regarded as an increase. Watson also failed to find any increase in uric acid in the blood or loss in alkalinity during acute attacks, while the excretion of uric acid was increased.

Garrod perfected a ready clinical method for the demonstration of uric acid in the blood or serum of gouty patients, commonly known as the "thread test."

One to two drachms of serum from fresh blood drawn from a vein are placed in a broad, flat dish, about three inches in diameter and one-third of an inch deep, and to it are added six minims 33 per cent. acetic acid to each drachm of serum. A couple of old but clean linen threads are submerged in the fluid and the vessel is allowed to partially evaporate at room temperature for thirty-six to sixty hours, when the threads will be found to have gathered minute crystals of uric acid, if any considerable trace is present in the blood. They may be washed in water and freed from any adherent phosphate and identified under the microscope. Garrod found that crystals begin to deposit on the thread when the serum contains 0.025 grain of uric acid per 1000, and become very numerous with 0.08 grain per 1000. The test must be carefully performed and may fail, especially from too rapid or prolonged evaporation, or from heating above 75° F. Serum obtained from a blister may be used, but not when obtained from the neighborhood of an inflamed joint.

Excess of uric acid in the blood is not, however, pathognomonic of gout, being absent in some undoubted cases (Duckworth), and being abundantly present in many other conditions, such as pneumonia (Salomon[2]), and other conditions with leucocytosis, emphysema, nephritis, severe anemia (v. Jaksch[1]), leukemia (Magnus-Levy[2]), and after a diet rich in nucleoproteids (Weintraud).

Alkalinity. The assumption by Garrod that an excess of uric acid in the blood is associated with diminished alkalinity has not been supported by late observations. Klemperer,[2] estimating the alkalinity by the content of the blood in CO_2, found no distinct variations from the normal in three cases during the attack. Luff, using Wright's method, also failed to find any loss of alkalinity during the course of an acute attack, and Magnus-Levy, using Lowy's method, failed to find any constant variations in a series of sixteen cases examined at various periods. Pfeiffer, v. Jaksch, and Drouin also failed to find any constant reduction.

From the recent studies of the chemistry of the blood in gout, it is evident that Garrod's theory of the nature of the disease must be abandoned.

OSTEOMALACIA.

The usual condition of the blood in osteomalacia appears to be that of moderate chlorotic anemia. Such cases are reported by Eisenhart, Tschistowitch, and others, while Seligman, in an advanced case, found 41 per cent. of Hb.

Red Cells. The red cells are usually normal or very slightly reduced. Shortly after abortion Tschistowitch's case showed 3,100,000 cells, but the numbers increased rapidly, later to oscillate between

3,400,000 and 6,800,000. One of Rieder's cases had 3,800,000 cells, another 4,900,000.

Leucocytes. The leucocytes have varied from subnormal to moderately increased numbers. The leucocytes are usually very numerous, an excessive proportion (maximum, 56 per cent.) having been found by Tschistowitch, while Ritchie observed moderate lymphocytosis. Myelocytes have been found by Neusser and by Tschistowitch.

Neusser[1] proposed to divide cases of osteomalacia into two groups, one showing myelocytes in the blood, the other showing high proportions of eosinophile cells, of which he had seen examples. High normal proportions of eosinophiles have been found by several observers, but not by others, viz., Fehling, Sternberg, and Chrobak, who were probably looking for a distinctly abnormal number. In Tschistowitch's case their numbers varied greatly during the year over which his observations extended.

Chemistry. The alkalinity usually varies between normal limits (Limbeck), but has been found both increased and diminished. Lactic acid has never been fully demonstrated in the blood. Peters claimed to have found in the blood a nitrifying micro-organism, cultures of which produced in dogs a condition resembling osteomalacia. This claim has not been established.

RACHITIS.

The state of the blood in rachitis varies with the extent and severity of the primary disease and is markedly affected by complications. Nearly all series of cases reported contain some examples of pronounced rachitis in which the **red cells** were nearly normal and the Hb very moderately reduced, and some observers, as Felsenthal and Morse, did not meet with severe anemia in any case. Nor does it appear that the anemia bears a very close relation to the extent of the disease, although as a general rule the advanced cases with enlargement of the spleen show greater anemia than do those without involvement of the viscera (Monti, Berggrun). It is agreed on all sides that the disease is not associated with any peculiar type of anemia, although the hyperemia of the bone-marrow might be expected to yield an unusually large number of nucleated red cells. Yet these cells are apparently not more abundant in rickets than in congenital syphilis (Monti), while Gundobin found the same grade and type of anemia in rachitic as in other poorly developed infants. Rachitis figures about equally with syphilis, etc., in the etiology of v. Jaksch's anemia.

The existence of rachitis in patients with normal blood raises a question as to the cause of the anemia usually found in the disease, and the conclusion seems justified that the anemia of rickets is largely referable to the general malnutrition of the patient and to a variety of complications, especially gastroenteritis. Moreover the pathological nature of the disease renders it extremely improbable that the lesion in itself has very marked effect upon the blood. So far as our present knowledge goes, the anemia of rickets is practically that of marasmus and gastroenteritis.

Simple chlorotic anemia is the usual condition of the blood of rachitic children. No other forms were encountered among the cases of Felsenthal and Morse, most of which were described as of moderate grade; and a few examples of the same type have been described by others. Although the red cells may be above 5,000,000, the Hb is invariably reduced, the color-index frequently falling as low as in primary chlorosis. Morse found an average Hb-index of 0.7, and Felsenthal obtained only 50 per cent. of Hb in one case with over 5,000,000 cells. The usual morphological changes in the red cells are to be observed, and a few nucleated red cells are nearly always to be found in the young subjects. The *leucocytes* in cases with mild anemia are usually found at the higher normal limits or distinctly increased. Rickets does not appear among Monti's cases of simple anemia without leucocytosis.

Grave secondary anemia is observed in many cases of rachitis in which there are almost always some serious complications. Although the progress of rachitic anemia is sometimes moderately rapid, the cause of the destruction of red cells is not always apparent. V. Jaksch saw the red cells fall from 1,600,000 to 750,000 within three months, while Luzet saw a reduction of 500,000 cells in three weeks. Usually the loss of cells occurs more slowly, and in the grave forms of anemia the impoverishment of the blood is usually attended with enlargement of the spleen and often of the liver.

The changes in the red cells are then similar to those of other forms of severe secondary anemia of chronic course, but poikilocytes and nucleated red cells are unusually numerous. The Hb-index varies. In a few instances, especially those of more rapid course, the Hb is deficient, but usually the Hb-index rises and may become abnormally high. Thus, Monti and Berggrun found 50 to 55 per cent. Hb with 2,350,000 cells. In all cases of this type there is leucocytosis.

Progressive pernicious anemia appears to be a relatively infrequent complication of rickets. In eleven cases of pernicious anemia in young children or infants, collected by Monti and Berggrun, rickets existed or was mentioned in only two, and one of these was complicated by congenital syphilis. The writer has had a similar experience, never having found the well-marked characters of progressive pernicious anemia in a rickety child.

Leucocytes. It has already been shown that in many mild cases of rickets the leucocytes do not exceed the normal limits for young infants. The slight excess over 10,000 seen in most of the mild cases reported is not, as Cabot points out, abnormal for infants. Felsenthal found 30,000 white with 4,000,000 red cells in one mild case in which no complication was noted.

In the severer cases leucocytosis is nearly constant, but does not usually exceed 30,000. Yet Luzet believes that there are all grades of the leucocytosis of rickets up to that of leukemia, where it is certainly difficult at times to distinguish the leucocytosis of a rachitic anemia from that of v. Jaksch's anemia. The lymphocytes are usually quite numerous, but not often excessive for the age. Morfound an average of 43 per cent. of lymphocytes in twenty rachitic children under two years of age. Distinct lymphocytosis was observed by Rieder, Weiss, Monti and Berggrun, and Morse, and, while probably not uncommon in young subjects, is without special signifiance.

Eosinophile cells are often relatively numerous. Morse found an average of 3 per cent. They may be distinctly increased, as Hock and Schlesinger found 20 per cent. in one subject and Weiss 16 per cent. in another, or they may be scanty (Rieder, Cabot).

The conditions leading to excess of leucocytes in rickets are not well understood. Limbeck refers the excess principally to the gastroenteritis which is often present. Whilst most cases with leucocytosis show hyperplastic splenitis, these two conditions are most probably separate effects of a common cause. Not all cases with enlarged spleen show leucocytosis, which is commonly absent in pronounced cases of "splenic anemia" in infants. Luzet, however, connects the excess of leucocytes with the hyperplasia of the spleen.

It seems not improbable that the *hyperemia of the marrow* may tend to discharge an unusual number of white cells into the circulation, a possibility which is favored also by the large number of nucleated red cells commonly seen, by the frequent presence of a few leucocytes with mitotic nuclei (Hock, Schlesinger), and by the nearly constant occurrence of myelocytes.

Chemistry. The specific gravity of the blood was reduced in proportion to the loss of Hb in the cases of Hock and Schlesinger. Even in patients who appeared otherwise in good health the specific gravity of the blood was distinctly reduced in twelve cases examined by Felsenthal and Bernhard.

MYXEDEMA.

The anemia of myxedema, in the majority of pronounced and untreated cases, is of the secondary chlorotic type, with slight leucocytosis, and of moderate grade. Bramwell observed well-marked anemia in twenty-six of thirty-three cases. Of twenty-three cases collected by Murray the blood was normal in seven, while in the cases of Cabot and in those of Kraepelin there was no anemia. The most severe anemia recorded was that of Le Breton's case, in a child of three years, with 1,700,000 cells, 65 per cent. of Hb, and 4500 leucocytes. Many other cases have shown a reduction of the red cells to about 3,000,000 (Bramwell, Putnam, and two cases of the writer). Kraepelin noted by careful measurements a *distinct increase in the diameters of the red cells* in four cases, and claimed this feature to be one of the typical symptoms of myxedema. The *chemical* examinations of the blood of these patients supported this conclusion by showing excess of solids. Le Breton also noted a similar condition in the blood of his case, but the patient was very anemic (1,700,000 cells). Vaquez found the majority of cells increased in size and varying from 8μ to 14μ in diameter. Treatment caused no increase in the number or change in size of the cells. Cabot failed to find any such changes in the red cells of three cases. When present it is probably referable to the increased venosity of the blood, which, according to Horsley, is commonly present in myxedema. The Hb is usually moderately reduced, but less than in most other forms of secondary anemia. In Le Breton's case the Hb-index was abnormally high (1.8), but since large nucleated red cells were present the condition of the blood seems to accord with that of pernicious anemia.

In two of Kraepelin's cases, while the Hb by Fleischl's method was not excessive (5,400,000 to 5,600,000 cells, 93 to 98 per cent. Hb), by the spectrophotometric method an abnormal quantity of blood pigment was demonstrated. The specific gravity was also abnormally high. In several other cases, even when the red cells were considerably deficient, the Hb-index was normal or increased (Bramwell). Putnam reports a few myelocytes in one case in which the blood was otherwise nearly normal.

Thyroid treatment has usually been followed by marked improvement in the blood. In six weeks Kraepelin's case gained 700,000 red cells, and nucleated red cells and leucocytes disappeared. Putnam observed polythemia (5,700,000) after six months' treatment. Marked anemia followed the administration of overdoses of thyroid extract in one of Bramwell's cases, but on smaller doses the patient did well. The course of the blood changes in this case was as follows:

	Red cells.	Hb.
October 28 (before treatment)	3,800,000	65 per cent.
November 28 (after acute thyroidism) . . .	2,600,000	54 "
December 21 (smaller doses)	3,800,000	68 "
January 13	4,300,000	70 "

Leucocytes are usually not increased, but in six of Murray's twenty-three cases there was leucocytosis. In uncomplicated cases without severe anemia it appears to be nearly always absent. The proportions of the various leucocytes are not altered. Cabot found 5 and 4.4 per cent. of eosinophiles in two cases, but Schmidt found only 1.5 per cent. of these cells. Putnam reports a few myelocytes in one case in which the blood was otherwise nearly normal.

Chemistry. Very numerous and elaborate studies of the chemistry of the blood in myxedema, and especially in thyroidism, have failed as yet to demonstrate the exact nature of this peculiar toxemia. Halliburton, in 1885, demonstrated mucin in the blood of thyroidectomized dogs, an observation which has been fully confirmed, and Levine has recently produced peculiar chronic toxemia by injection of mucin in animals after partial thyroidectomy, although similar injections in healthy animals were innocuous. Yet in the human subject an excess of mucin in the blood has not been demonstrated.

The chemical analysis of Kraepelin's cases, by Schneider, showed the exaggerated effects of chronic venosity of the blood, a condition first noted clinically in myxedema by Horsley. Here there was con-

siderable increase in specific gravity of the blood (1.0625 to 1.0636) and of the serum (1.0317 to 1.0329), while the dry residue of whole blood, serum, and red cells was also increased.

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CHAPTER XIX.

NERVOUS AND MENTAL DISEASES.

MANIA, GENERAL PARESIS, MELANCHOLIA, DEMENTIA, EPILEPSY.

THE blood of inmates of insane asylums has been the subject of much careful study.

In 1873 Sutherland examined the blood of 149 insane patients, finding in four of them grave secondary anemia with leucocytosis. Excess of leucocytes occurred in many other milder or earlier cases.

McPhail contributed the first scheme of the blood changes in *general paresis*, finding from a study of fifteen cases that:

1. The Hb was moderately low on admission (62.70 per cent.), improved for a time, owing to the better hygienic conditions prevailing in the institutions, but fell (52.66 per cent.) in the terminal stages of the disease. 2. The red cells diminished steadily with the progress of the disease, reaching in one case 3,400,000. 3. Leucocytosis (minimum, 12,700) was noted in all cases, progressed with the disease, and reached a well-marked grade in the terminal stages, 27,700 to 36,600.

Lewis, Steele, Seppili, and others obtained results very similar to the above and added many new details. Smyth examined the blood in cases of melancholia, epilepsy, general paresis, and secondary dementia. He found the red cells as a rule below the normal, while the Hb was reduced in much greater degree. The average results obtained were as follows:

	Cases.	Hb.	Red cells.	Specific gravity.	During convulsions.
Melancholia	10	69.7	4,684,000	1.0572	
Epilepsy	50	62.8	4,520,000	1.0568	1.0596
General paresis	40	68.7	4,700,000	1.0605	
Secondary dementia	12	53.7	4,070,000	1.0612	

The more marked anemia, with high specific gravity, of secondary dementia, and the increase of specific gravity after epileptic convulsions, which was not invariable, are specially worthy of note.

Winckler also found a distinct loss of Hb, especially in the depressed forms of insanity. Paroxysms of mania or of melancholia caused a loss of both cells and Hb, which was restored after the recovery from the attack. Terminal dementia was associated with a progressive anemia. Capps examined twelve cases of general paresis in which the red cells never fell below 4,000,000, while never reaching 5,000,000 (average 4,789,000). The Hb varied between 73 and 92 per cent. (average 85 per cent.), and the specific gravity between

1.058 and 1.066. Convulsions appeared to concentrate the blood. Jelliffe, working at the Binghampton State Hospital, altitude 400 metres, found moderate polycythemia in fourteen of seventeen cases, but the Hb was usually reduced, in one case to 52 per cent. The specific gravity ranged from 1.047 to 1.060. Langdon and Bamford, at the Hudson River State Hospital, obtained in a reasonably short time twenty very anemic cases of melancholia, with red cells between 2,000,000 and 3,000,000, Hb between 60 and 85 per cent.

Treatment by bone-marrow rapidly improved the condition of the blood. Severe anemia, red cells 2,500,000, Hb 25 per cent., is also reported by Howard in a case of terminal dementia, while in Steele's thirty-five cases of melancholia the average of red cells was 3,000,000, of Hb 75 per cent.

The Leucocytes. In early cases there is usually no leucocytosis, and the uniform tendency to leucocytosis observed by McPhail has not been found by all subsequent observers. Smyth reported an excess in many cases, but without relation to the symptoms of the disease.

Most of Capps' patients had leucocytosis, as did also the very anemic subjects examined by Langdon and Bamford, while in the tables of Jelliffe and Somers leucocytosis was absent in the majority of cases. Leucocytosis is more frequent when there is anemia, in the last stages of the disease, and it has been found rather more frequent in the more acute forms of insanity and in terminal dementia. Somers found an average of 8315 leucocytes in nineteen cases of mania; 7947 in nineteen cases of melancholia; 10,473 in nineteen cases of dementia, and 8800 in five cases of general paresis. In sixteen cases of epilepsy Kuhlmann obtained distinct leucocytosis in only one (14,000). Pease followed the leucocytosis long enough to show that it is often transitory.

The proportions of the *various forms of leucocytes* show no constant or significant variations. When the total numbers are increased the polynuclear forms are usually most affected. *Lymphocytosis* is frequently observed, especially in epilepsy. The eosinophiles show irregular variations, being absent in some cases, reaching high normal figures in others, while Roncorini found a marked excess, up to 25 per cent., in maniacal cases. Krypiakiewicz also found an increase of these cells in the more acute forms of insanity, and comparatively low proportions in the chronic forms. Capps could not find that the restlessness of the patient was always connected with any increase in eosinophiles.

To recapitulate the results of the above studies, it has been shown that the common forms of insanity may develop in anemic subjects or in those whose blood is normal. The disease may run its course without anemia, but usually the state of the blood accords with the general state of nutrition. In some cases, especially of melancholia, the blood suffers more than the general nutrition.

Leucocytosis is frequently observed, especially in anemic subjects, in late stages, and after convulsions. Convulsions may concentrate the blood and induce leucocytosis. Acute mania is not associated with any specific changes in the morphology of the blood.

BERIBERI.

This form of infectious neuritis is often associated with severe or even pernicious anemia (Spencer), but in the cases which reach America the anemia is usually moderate and of the chlorotic type. In three recently imported cases of the disease, each with moderate fever (100° to 101.5° F.), the red cells ranged between 3,000,000 and 3,500,000, and were very deficient in Hb, while showing no other changes, except for the presence of microcytes. Leucocytosis was absent and the eosinophiles were not increased. Cabot records an afebrile case with 3,900,000 red cells, 48 per cent. Hb, and 7800 leucocytes.

Daubler found normal numbers of red and white cells, but increase of fat, in the blood of three cases.

Numerous attempts to demonstrate the infectious agent in beriberi are thus far negative. Various bacteria have been demonstrated in the blood postmortem. The latest contribution is that of Fajardo, who believes that he has seen in the red cells of fifty-nine cases a *protozoon* resembling the parasite of Texas fever, but smaller and more difficult to stain.

Rost is reported to have found a diplobacillus in the rice eaten by natives suffering from beriberi, and the same bacillus in the blood, cerebrospinal fluid, and inflamed nerve sheaths of beriberi patients.

Stanley planted 1 c.c. of blood in each of thirty cases and found no growth, while in the smears there was no trace of the hematozoon described by Fajardo. Affleck also reports negative bacteriological studies of the blood.

CHOREA.

The coincidence of St. Vitus' dance and chlorosis was such a frequent clinical picture that the older writers regarded the anemia as one of the essential causes of such nervous manifestations. Although this view has been proven untenable it remains true that anemia and chorea have many common antecedents, that the blood in chorea usually shows a slight impoverishment, and that anemia is a very frequent predisposing cause of chorea.

Burr found moderate chlorotic anemia in all of thirty-six cases, but any severe grade was always referable to complications. Leroux found 5,000,000 cells in two cases, between 3,400,000 and 4,800,000 in five, and 2,200,000 red and 89,000 white in an infant in which the disease was complicated by furunculosis. Zappert's four cases showed moderate reduction in red cells, 3,900,000 to 4,500,000. Litten has recorded two fatal cases arising in the course of pernicious anemia. Cabot refers to twelve cases showing no abnormality except eosinophilia.

The leucocytes in uncomplicated cases have been found normal in numbers, but often with excess of eosinophile cells. Zappert counted 630 to 1360 (8 to 19 per cent.) of eosinophiles in four cases.

The *bacteriological examination of the blood* during life has thus far been negative, although Leredde obtained the *staphylococcus albus* from the blood of one case complicated by endocarditis. The blood of the cadaver has been found to contain various micro-organisms, Triboulet having three positive results in fifteen cases. Bacteriological studies of the blood and viscera favor the belief in the identity of origin of rheumatism and chorea.

Functional nervous diseases, such as **hysteria**, **neurasthenia**, **hypochondriasis**, and **tetany**, do not give rise to anemia, but numerous associated conditions may lead to impoverishment of the blood. Cabot and Reinert report mild grades of chlorotic anemia in hysteria and neurasthenia. In neuritis Cabot reports chlorotic anemia with marked leucocytosis (16,000 to 28,000) in a febrile case in a young subject, while in four of six cases of afebrile alcoholic neuritis the leucocytes were slightly increased.

GRAVES' DISEASE.

Anemia does not appear to be essentially connected with exophthalmic goitre, as some cases show nearly normal cells and Hb (Oppenheimer). Bramwell reports anemia, grade not stated, in only twenty-seven of sixty-four cases. Yet many patients suffer from a form of anemia closely resembling chlorosis, which is sometimes quite severe. Thus Zappert records two cases with 2,800,000 and 2,700,000 cells, and 32 to 30 per cent. of Hb. The writer has examined the blood in several such cases, in one of which the majority of red cells were distinctly undersized.

The term "*thyroid chlorosis*" is applied by Capitan to cases of chlorosis associated with enlargement of the thyroid. Referring to Hayem's report of twenty-nine goitres among thirty-five chlorotic patients, Capitan finds reason to believe that one type of chlorosis is referable to thyroid intoxication. Some years ago the writer was struck by the peculiar clinical type of chlorotic anemia observed in two elderly women suffering from Graves' disease, but has not since encountered such cases.

The leucocytes, in the absence of complications, are normal or diminished. Neusser and Cabot have noted relative lymphocytosis.

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PART V.

GENERAL DISEASES OF VISCERA.

CHAPTER XX.

THE HEMOPOIETIC SYSTEM.

THE LIVER.

Relation of its Functions to the Blood. Although the chief functions of the liver in the secretion of bile and the elaboration of absorbed food products are directly concerned with the maintenance of the blood albumins, specific effects of inhibition of these functions have not yet been traced in the blood. These effects are doubtless seen in the anemia of advanced cirrhosis, but are even then not to be distinguished from similar changes in other secondary anemias.

The function of transforming into bilirubin and removing from the system the pigment of disintegrated red cells is being constantly exerted in health and disease. When from any cause the excretion of this product is obstructed, as in acute yellow atrophy, or the demands upon the function are excessive, as in pernicious malaria, either hemoglobin or its derivative, bilirubin, accumulates in the blood, giving hemoglobinemia or jaundice. In the former case the content of the liver in hematoidin and hemisiderin is an accurate index of the grade of blood destruction. There is as yet no proof that the liver plays more than a passive part in the destruction of blood.

The embryonal function of the liver as a depot of forming red cells is not infrequently retained until after birth, and there is reason to believe that the renewal of this function may sometimes give peculiar character to the anemia of infants described by v. Jaksch.

Effects of Bile upon the Blood. A globulicidal action of bile was first demonstrated experimentally by Hunefeld (1840), while the fact that the biliary acids are the active agents in dissolving red cells in jaundice was shown by v. Dusch (1854), and fully established by many later studies. V. Dusch not only demonstrated the power of bile to dissolve red cells, but claimed that it may also dissolve leucocytes and even liver cells, a property noted also by Rywosch. The relative globulicidal power of the various salts of biliary acids was fully tested by Rywosch, who found that sodium ch[illegible] and taurocholate dissolve red cells when in a concentration of 1 to 700, or 1 to 600, that Na-cholate is only moderately active at 1 to 200, while Na-glycocholate destroys red cells only when in a solution of 1 to 50. In the process of solution the red cells first lose their central depression, become variously deformed and finally dissolved, leaving no detritus. These changes have been observed only in the test-tube, but in the blood of most cases of jaundice the process appears to differ from that of other forms of hematocytolysis.

The resistance of the red cells was found by Limbeck, in all cases of jaundice, to be distinctly increased, the cells not dissolving in solutions of more than 0.32 per cent. NaCl (normal 0.46 per cent.). This change he referred to the destruction of less resistant cells and a chemical union of Hb and stroma brought about by the action of bile salts.

Vaquez and Riberre find that the red cells begin to show increased resistance on the fourth day of jaundice, which continues until in prolonged cases they may fail to dissolve in 0.24 per cent. NaCl. They refer this peculiarity to the development of antilysins, which are present in the serum, since this serum, added to normal red cells, prevents the usual solution by bile.

An increase in specific gravity of the blood was observed by Grawitz, following the injection of bile in animals, and referred by him to transudations excited by some specific action on the part of the bile. Limbeck also found a relative decrease in volume of the serum in obstructive jaundice, as well as a lower percentage of chlorides. At the same time the volume of the red cells was increased.

Coagulation is hastened, according to Rywosch, by the presence of one part of Na-chenocholate or taurocholate in 500 of blood, but entirely inhibited when the proportion reaches 1 to 250.

The presence of bile pigments has no deleterious action on the blood, although Rywosch found that injections of pure bilirubin, while not affecting the blood, produce mild general toxic symptoms.

Diseases of the Liver.

Jaundice. In the human subject clinical observations have shown that small traces of bile are demonstrable by the greenish-yellow color of the serum of very mild cases in which, however, there are usually no other changes in the blood. In severe cases, when considerable quantities of bile are present in the circulation, many of the effects observed in the experimental study of jaundice become apparent. Any tendency toward concentration which the presence of bile may exert is seldom seen clinically, as the majority of severe cases of jaundice exhibit some reduction of red cells. This anemia is usually referable to pre-existing conditions, but in very severe cases actual destruction of red cells may be traced in the dried specimen. A tendency to very *rapid crenation* and total *absence of rouleaux* have been observed by Grawitz. In the blood of icteric infants Hofmeier also noted deficiency in the formation of rouleaux, which, however, are normally less numerous than in the blood of adults.

Evidences of *solution of red cells* were found by Silberman[1] in the blood of many jaundiced infants. These consisted in the presence in the blood of fragments of red cells, and in the liver and spleen of many phagocytes inclosing red cells and blood pigment. Many other degenerative changes in the red cells were noted, but they were not necessarily connected with the jaundice. In severe cases examined by the writer, besides the ordinary signs of anemia, there were in the blood many very pale and some fragmented red cells, and in the viscera deposits of pigment equal to those of pernicious malaria. After death reddish acicular crystals (bilirubin ?) were sometimes found in the blood.

A *polycythemia* referable to the concentrating effect of bile in the blood has not often been reported. Yet Becquerel and Rodier reported the highest figures in their experience as occurring in a case of jaundice, and later observers have abundantly shown that well-marked jaundice markedly increases the specific gravity of the blood in proportion to the intensity of the jaundice. Grawitz saw the gravity rise from 1.050 to 1.061 in a severe case, but does not report the number of red cells, while Limbeck and Van Noorden[1] found the dry residue of the blood to be 22 to 25 per cent. in several cases. The gravity of the serum was unaffected by the presence of bile in Hammarschlag's twelve cases, but in Limbeck's two cases the serum was poor in chlorides. The results of Limbeck's chemical analyses indicate that the volume of red cells in jaundice is much increased.

The *alkalinity* of the blood is in mild cases unchanged (Limbeck), but in acute yellow atrophy, de Renzi, using Reale's method, found a neutral reaction, while an acid reaction was present in severe catarrhal jaundice and in suppurative hepatitis with jaundice.

The leucocytes, in simple catarrhal jaundice, have usually been found unaffected, but in Grawitz's experience an increase has been common and occasionally the leucocytosis has been very marked (38,000 to 40,000). It is probable that different observers refer to different types of cases.

Cancer of Liver. A large proportion of the carcinomata of the liver being primary in the stomach, the anemia of this condition is frequently grafted upon that of gastric cancer. The reports of Wlajew include one case of secondary pernicious anemia with only 850,000 red cells, and none showing over 3,000,000, while the leucocytes ran between 20,000 and 40,000, indicating that this anemia is usually severe. The writer's experience at autopsies in these cases accords in general with Wlajew's results, as marked emaciation and distinct oligemia are usually prominent. The sudden onset of severe jaundice in many of the cases suggests the probability that the blood may at times suffer concentration.

Cabot's series of seventeen cases showed well-marked anemia, cells under 3,000,000 in only two, while in ten there were over 4,000,000.

The *leucocytes* are usually increased in cancer of the liver, but the increase is often intermittent. Of nineteen cases Cabot found only six with white cells under 10,000, and in none of these six was the examination repeated. Alexander found leucocytosis in two cases, but only after repeated examinations. The grade of leucocytosis is usually moderate. The fact that when attacking the liver most carcinomata are already generalizing, the tendency toward rapid growth in the organ, and the early formation of further metastases, are some of the factors which account for the frequency of leucocytosis in hepatic cancer.

Hypertrophic Cirrhosis with Jaundice. More severe alterations of the blood have been observed by Hayem in a case of hypertrophic cirrhosis in which the red cells fell below 2,000,000, with increased Hb-index and leucocytosis. Severe anemia was also present in two cases verified at autopsy and reported by Cabot, but in one only 2400 leucocytes were found.

Hanot and Meunier have insisted upon the presence of leucocytosis in this form of cirrhosis of liver, finding therein evidence of the *infectious nature of the disease.* They found from 9000 to 21,800 leucocytes in five cases. Lukachewitch also found 20,000 white cells in another case, and Auche reports in three cases intermittent leucocytosis, 12,400 to 18,600 cells. Bacteria, usually *staphylococcus pyogenes aureus,* have been found in the blood of "infectious jaundice," sometimes with hypertrophic cirrhosis, by Netter.

Cholelithiasis. Considerable interest attaches to the bacteriological examination of the blood in cholelithiasis from the recent discovery of bacteria in the blood, in several cases, usually in those marked by intermittent fever. *Staphlycoccus pyogenes aureus* has been isolated in two cases by Netter; in three cases, once with the colon bacillus, by Sittmann; and in the pus of metastatic abscesses by Gilbert and Girode, Brieger, and many others. The *pneumococcus of Frankel* and *streptococcus pyogenes* were obtained from the blood in one case each, both complicated by hepatic abscess, by Canon and by Zancarol, while a small bacillus, not identified, was obtained from a complicating hepatic abscess and from the ulcerated heart valves, by Netter and Martha.

The anemia in these cases varies with the previous condition of the patient and with a great variety of associated lesions.

The behavior of the leucocytes is of interest, and may be of value in differential diagnosis. Biliary colic may supervene in severe attacks without producing any leucocytosis. The onset of jaundice is sometimes accompanied by moderate leucocytosis, at other times fails to cause any increase of white cells. The difference probably depends upon the grade of inflammation excited by the gallstones, etc. Here as elsewhere one may expect no leucocytosis with simple catarrhal inflammations, but with active suppurative lesions leucocytosis may reach a pronounced degree (up to 50,000, Cabot). As some of these lesions run into abscess of the liver, or pyemia, even this high figure is probably not the limit of the leucocytosis.

Abscess of Liver. The lesion in abscess of the liver may follow one of several types:

1. There may be a large number of small or miliary abscesses throughout the liver, filled with creamy or muco-pus. These abscesses

arise from extension of suppurative cholangitis. The leucocytosis is probably a continuation of that observed in cholangitis, but there appear to be no direct observations on the blood of this condition.

2. There may be one or two large cavities filled with creamy or stringy pus, and well shut off from the liver tissue by mucous or pyogenic membrane. The writer has seen such an abscess run its course with little or no increase of white cells in the blood, and the leucocytosis is usually slight. In one adult case, the day before one-half litre of pus was evacuated at operation, with temperature 101° F., the blood showed 11,000 leucocytes, 52 per cent. of which were mononuclear, 48 per cent. polynuclear. A previous examination had also failed to show distinct leucocytosis.

3. Large necrotic abscesses with actively suppurating walls are practically always attended with marked leucocytosis, up to 50,000, but this increase may be *distinctly intermittent.*

Acute Yellow Atrophy. Cases recorded by Grawitz and Cabot showed 5,150,000, and 5,520,000 red cells, and 12,000 to 16,000 leucocytes. The exact limits of this disease are as yet imperfectly determined, but in three cases seen by the writer there was in each a moderate leucocytosis, 15,000 to 21,000. Two of these occurred in parturient women at Sloane Maternity Hospital, and showed the usual lesions in the liver. In another at Roosevelt Hospital, the disease lasted three weeks, and the inner four-fifths of the hepatic lobules were necrotic. The red cells were not counted, and the absence of rouleaux and the early crenation mentioned by Grawitz were not noted. In two cases bacteriological examinations of the blood during life, by Sittmann, were negative, but Vincent obtained the colon bacillus from the blood of one case during life.

Echinococcus Cyst of Liver. Hayem classes the echinococcus cyst of the liver as one of the conditions leading to leucocytosis and excess of fibrin in the blood. Auche in one case ventured to rule out the diagnosis of hypertrophic cirrhosis from the absence of an excess of leucocytes, the patient showing an echinococcus cyst at autopsy, while Wlajew found no change in the blood in a case of hydatid cyst of the liver. Neusser, Lépine, Dargein, and Seligman all report cases of echinococcus cyst of the liver with leucocytosis varying from 15,000 to 28,000 and eosinophile cells from 12 to 57 per cent. In some instances there was polycythemia.

Cirrhosis of Liver. In the early stages of the disease, when digestion is not much impaired and there are neither jaundice nor hemorrhages, the blood is but little altered. Almost invariably the Hb is deficient. During the progress of the disease the blood steadily deteriorates, giving some of the most typical examples of secondary anemia. Abundant cause of the anemia is found in all cases in the disturbance of the functions of the liver and stomach, and in other cases by the complications of the particular type which the disease follows. In slowly progressing cases without special complications the blood commonly shows between 3,000,000 to 4,000,000 cells, 55 to 75 per cent. of Hb, and little or no leucocytosis.

In Haan's twenty cases the Hb ran between 60 and 110 per cent., while the Hb-index was uniformly high, above normal in eleven cases.

The ordinary **progress of the anemia** is perhaps most affected by *profuse hemorrhages*, after which the blood shows the usual reduction of red cells and Hb, with nucleated red cells and leucocytosis. Single hemorrhages may occur in subjects which are only slightly anemic, and the blood is then rapidly restored. In the later stages of the disease single or repeated hemorrhages if not at once fatal often lead to very grave secondary pernicious anemia.

Ascites, although draining the blood of much albumin, is commonly found with lesser grades of chlorotic anemia. It is not uncommon to find, in well-advanced cases requiring paracentesis, 4,500,000 to 5,000,000 red cells and Hb above 65 per cent. Grawitz believes that the ascitic transudate leads to some concentration of the blood and the masking of anemia. Such a condition is strongly suggested at times by the disproportion between the anemic appearance of the patient and the slight changes demonstrable in the blood. The *effects of paracentesis* vary. Limbeck observed a case of Laennec's cirrhosis, with extreme ascites, in which the removal of eighteen litres of fluid raised the red cells after twenty-four hours from 3,280,000 to 5,160,000. Three days later they fell to 3,540,000. Osterspey noted the same result after several tappings of the same patient. On the other hand, Grawitz saw the red cells fall from 4,700,000 to 4,300,000 shortly after tapping, but the first examination came three days before the operation. The results of tapping may very well vary with the immediate effects upon the circulation, with the state of the kidneys, and with the reappearance of the ascites.

Jaundice, if mild, has no demonstrable influence on the blood of cirrhosis; nor do the ordinary chronic forms of moderately severe jaundice appear to be accompanied by any special alteration in the blood except a tendency toward leucocytosis.

In most of the jaundiced cases reported by Hayem, Cabot, and others there was a moderate leucocytosis, reaching 20,000 or more. Haan, in nine cases with jaundice, found the leucocytes to vary between 2100 and 20,900, and in eleven cases without jaundice between 16,600 and 38,000. Severe jaundice usually occurs in advanced and anemic cases, or in the hypertrophic forms. The ordinary cirrhotic process in the liver is unaccompanied by leucocytosis, which, however, may frequently arise from many complications or accidental causes. Thus Hayem, Osterspey, Cabot, and others found no leucocytosis except from complications, but Grawitz, Rosenstein, and Wlajew refer to cases with moderate leucocytosis without giving details.

DISEASES OF GASTRO-INTESTINAL TRACT.

Esophagus.

Stenosis of the esophagus, when extreme, by diminishing the quantity of fluid ingested, has been shown to lead to marked concentration of the blood. Van Noorden[2] refers to two cases of cancer of the esophagus in which the dry residue of the blood reached 26.5 and 27.3 per cent. (normal 21 to 22 per cent.). When to this condition of concentration is added the cachexia which belongs to carcinoma and to starvation, a marked degree of oligemia may result, approaching that of pernicious anemia. A distinctly reduced quantity of blood was noted at autopsy in one of two cases of epithelioma of the esophagus, observed by the writer at Roosevelt Hospital, while in the other, in which death resulted from pulmonary extension and pneumonia, the stenosis was not extreme. These same factors may perhaps be responsible for some relative increase in the numbers of red cells which has been observed in a few cases. The red cells in reported cases have been above normal, or very slightly reduced, as in other forms of cancer, or greatly reduced, as in the severe anemia of cancerous cachexia.

Comparing his own results in a single case with those of Escherich, Pee, and Osterspey, Rieder concluded that the cachexia of epithelioma of the esophagus differs from that of carcinoma in other regions in the absence of leucocytosis. Reinbach also reported two cases with relative lymphocytosis. Cabot and Hoffman, however, report several cases with moderate leucocytosis. In the writer's cases there was *antemortem* increase of white cells. There has been no attempt to refer these varying results to the special causes of leucocytosis to which they are probably due.

Diseases of the Stomach.

General Considerations. Since the blood depends for its supply of albumins chiefly upon the functional activity of the stomach there is an intimate relation between the condition of the blood and the activity of digestion and absorption in this organ.

1. Transitory functional disturbances of the stomach usually fail to notably alter the blood, but persistent vomiting with hyperacidity has resulted in distinct concentration of the blood and reduction of chlorides both in blood and urine.

2. While total withdrawal of food for a short period causes comparatively little change in the blood, a long-continued failure of digestion or absorption is one of the most efficient causes of anemia. It has already been shown that organic lesions of the stomach are prominent among the pathological findings in cases of pernicious anemia, including chronic gastritis, atrophy of gastric mucosa, stenosis of the pylorus, and cancer. All the lesser grades and types of anemia are observed, with even greater frequency, to be associated with gastric lesions, while the anemia of marasmic infants is doubtless largely owing to failure of digestion and absorption, without organic lesion in the stomach. Meynert's theory of chlorosis further illustrates the well-founded belief in the importance of normal digestion in maintaining the normal condition of the blood.

3. The stomach is a frequent site of loss of blood by hemorrhage. If, as is indicated by the studies of Quincke and of Dettwyler and Silberman,[2] anemia or hemoglobinemia are strong predisposing causes

of ulceration of the stomach and duodenum, there is in the interaction of these two conditions a "circulus vitiosus" which readily explains their frequent association in the same subject, and the very severe grades of anemia which thereby result. Smaller and more frequent bleedings also greatly aggravate the anemia which accompanies ulcerating carcinoma.

4. Much has been written about the effects of gastrointestinal toxemia, but the chief source of autotoxic agents is without doubt the intestine.

The Blood in Hunger.—Muhlman has contributed an exhaustive critique of the extensive literature on the blood in hunger, drawing the following general conclusions: The specific gravity is uniformly increased. The volume of blood diminished 17.5 per cent. after moderate periods of abstinence from food, and as much as 37 per cent. after extreme periods. The red cells are not much changed, although sometimes slightly increased, until 15 per cent. of the body weight is lost, after which they diminish steadily until death. A maximum loss of 32 per cent. has been observed on the twenty-eighth day. Some observers, however, found polycythemia at all periods. The leucocytes fall 20 per cent. until 20 per cent. of the body weight is lost, after which they tend to increase, and are usually found above normal. The lymphocytes diminish steadily from the first, while the polynuclear cells diminish at first, but later reach normal numbers.

Grawitz finds that hunger in man tends to increase the Hb and red cells. A deficiency of albumins and fats in the blood reduces the albumins of the serum, before any change in Hb is observed, but if this deficiency is continued anemia with loss of Hb follows.

Special Diseases of the Stomach.

Acute Gastritis, Dyspepsia, Hyperacidity. These conditions, if not prolonged, have no tendency to produce anemia. If vomiting is excessive the cells and Hb may be increased. The leucocytes are sometimes increased, sometimes normal or diminished. Their numbers may indicate the intensity but not the etiology of the inflammation.

Chronic Gastritis. Chronic gastritis may be tolerated for long periods without leading to any marked anemia, as the writer has often observed in cases addicted to alcohol. When the patients become disabled anemia of the secondary chlorotic type is usually found to be associated with loss of flesh. The leucocytes are low and lymphocytes usually prevail (Blindeman, Cabot, Hoffman). Digestion leucocytosis may be diminished and is sometimes absent, as in cancer.

Associated with chronic gastritis and fibrous stricture of the pylorus, the writer has twice observed slowly progressive anemia lasting four and five years. The changes in the blood were first chlorotic, later intermediate, and, finally, those of pernicious anemia without many megaloblasts in the blood, but with typical changes in the marrow. Much more acute and typical cases of pernicious anemia have been referred to chronic gastritis (Pepper, Stengel).

Dilatation of Stomach. In cases of gastrectasis from chronic gastritis or stenosis of pylorus the blood does not differ from that of simple uncomplicated chronic gastritis. Although the patients become cachectic and lose flesh, the blood usually fails to show corresponding anemia. In the cases reported by Reinert, Cabot, Blindeman, and others the red cells and Hb were normal or increased or but slightly reduced. It is generally agreed that oligemia exists in these cases, but its origin is not clear, and it is by no means evident that the concentration of the blood is affected, as Kussmaul suggests, by excessive vomiting. Defective absorption is probably a more important factor.

The Blood in Gastro-intestinal Diseases of Infants. Acute gastro-enteritis occurring in healthy infants usually tends to concentrate the blood, but these effects are demonstrable only in severe cases. Felsenthal and Bernhard, in twenty cases, found uniform polycythemia reaching 7,500,000 cells, and usually moderate leucocytosis. Hock and Schlesinger found a moderate increase in specific gravity in very severe but not in ordinary cases. On the other hand, in an anemic rachitic infant with diarrhea the Hb fell in one week from 80 to 60 per cent., indicating that a pre-existing anemia may be aggravated by such attacks.

In chronic gastro-enteritis there may be progressive anemia and emaciation, as in Hayem's case, in which after two months the red cells numbered only 685,000, leucocytes 18,910. On the other hand, Monti and Berggrun report a case lasting six weeks in which the blood of the emaciated subject showed 4,100,000 red cells and 65 per cent. of Hb. In most of Felsenthal's cases the Hb rose above 65 per cent. only when there was marked polycythemia, while moderate leucocytosis was the rule.

The grade of anemia observed in *marasmic infants* with mild gastro-intestinal disturbance is very variable, and its different phases illustrate all the stages of secondary anemia. It is usually characterized by relative or absolute lymphocytosis.

The behavior of the leucocytes varies greatly, being influenced by the age of the patient, the type of the disease, and the occurrence of complications, such as vomiting and severe diarrhea, which concentrate the blood, and hemorrhage with anemia.

Japha, in a series of miscellaneous hospital patients under one year of age, found the leucocytes to vary greatly, while the average was 13,500, of which 42 per cent. were polynuclear cells.

In dyspepsia, simple gastro-enteritis (dyspeptic diarrhea), and infantile atrophy he found no changes in the proportions of leucocytes and only the ordinary variations in their numbers.

In follicular enteritis and cholera infantum with distinct anatomical lesions of the intestine there was usually moderate or considerable polynuclear leucocytosis. Similar results have been obtained by Gundobin, d'Orlandi, and Knox and Warfield. Most of these observers include in their series cases of pronounced ulcerative colitis, in which the usual polynuclear leucocytosis was often marked.

On the other hand, several observers have encountered distinct, absolute, and slight or marked relative lymphocytosis in the milder intestinal disorders of infants.

Fischl found considerable leucocytosis with 60 per cent. or more of lymphocytes to be the rule in his cases. Hock and Schlesinger, in the above-mentioned case, found 38,000 leucocytes, the majority of which were large lymphocytes. Stengel and White, in an acute case with blood-streaked mucous stools, counted 70 per cent. of lymphocytes among 28,000 cells. In two cases of cholera infantum reported by Weiss the lymphocytosis approached the grade of lymphatic leukemia.

Ulcer of Stomach. The state of the blood in ulcer of the stomach is extremely variable, and it is not always evident what conclusions to draw from the results of its examination. From the very numerous cases reported in greater or less detail by Leichtenstern, Laache, Haeberlin, Oppenheimer, F. Müller, Schneider, Reinert, Osterspey, Cabot, Rochemont, and others, as well as from the writer's own observation, these changes seem to fall in some rather distinct classes.

1. Some cases appear to have suffered no permanent change in the composition of the blood, but from their anemic appearance it is probable that the total volume of blood has been reduced, without marked alteration in its quality

Most of these patients have never sustained a large hemorrhage, or a former loss of blood has been replaced through the active regeneration which is often seen in this condition. Müller and Oppenheimer have referred especially to such cases, while Grawitz demonstrated in one patient that the blood was decidedly hydremic, the dry residue of the serum being only 8.56 per cent., while that of the whole blood was 19.75 per cent., and the red cells numbered 4,340,000. This patient was recovering from more severe anemia and still gave anemic heart murmurs. A single chemical analysis, while most important, cannot with certainty be applied to all these patients, however, and several of Oppenheimer's reports indicate that the blood may be entirely normal in some cases of gastric ulcer. It does not seem probable that vomiting or other causes of concentration of the blood are important factors in the majority of these cases.

2. The *usual state of the blood* is one of marked secondary chlorotic anemia, with little or no leucocytosis, the presence of which depends largely upon the manner of feeding. While the regeneration of the blood after hemorrhage is often very rapid, it is seldom complete while the patient remains in the hospital, and there seems to be some influence, possibly found in a diminished digestive power in the stomach, which causes the anemia to persist.

The writer followed one male patient for five years after a dangerous hemorrhage, but although all local and other general symptoms had disappeared, the blood never showed more than 75 per cent. of Hb.

3. The bleedings of ulcer of the stomach furnish some of the most remarkable examples of post-hemorrhagic anemia, the characters of which have been described. Surviving patients seldom show less than 1,000,000 cells, but from this point may improve rapidly, with low Hb-index, normoblasts, and leucocytosis. Cases with cells under 3,000,000 have usually suffered from hemorrhages, which if frequently repeated may lead to grave anemia with rising Hb-index and increasing diameters of red cells. The lowest specific gravity of the blood (1023) encountered by Grawitz in his studies was in a case of gastric ulcer with 400,000 red cells.

Leucocytes. Leucocytosis of moderate grade follows hemorrhage or local inflammation. In one case with marked leucocytosis the writer found two perforated ulcers, one opening into a large cavity in the liver, the other connected with an abscess of the liver. In quiescent periods, with rectal feeding, there is usually hypoleucocytosis, while the change to feeding by the stomach may excite considerable digestion leucocytosis, as in Cabot's case, in which the first meal raised the white cells from 4000 to 15,000. These features may be of diagnostic value when cancer is suspected, but should be interpreted with caution. Digestion leucocytosis has been absent in ten cases of ulcer reported by Hoffman, Hassman, and Schneyer. In three of these there was stenosis of the pylorus.

Cancer of Stomach. The usual course of the anemia of malignant neoplasms is varied by the peculiar conditions connected with a tumor of this organ, and by the extent and character of the new growth. In the reports of some 200 cases to be found in the literature there has been no systematic attempt to classify the types of anemia with reference to the character of the tumor, and while it appears by no means certain that such a classification would be of clinical value, there are, nevertheless, certain features of the growth and complications of these tumors which chiefly determine the state of the blood.

1. *Some cancers of the stomach exist for considerable periods without leading to marked impoverishment of the blood*, and there is no necessary cause of anemia in the early stages of most of those which do not bleed, ulcerate, or contract the pylorus. When chronic gastritis or ulcer precede cancer, the pre-existing anemia suffers at the time no appreciable change. Accordingly, it is a common experience to find in the blood at this time no distinct indication of the presence of a malignant tumor in the body. At this time the general symptoms are more reliable diagnostic signs than is the state of the blood. Moreover, if vomiting is a prominent early symptom, an initial anemia may be masked by temporary concentration of the blood. Cabot reports over 4,000,000 red cells in 64, and over 5,000,000 in 25, out of 114 cases. Price-Jones followed a case which at first showed 6,270,000 red cells, 50 per cent. Hb, but one month later 3,750,000 and about the same amount of Hb, 48 per cent.

The *first sign* of affection of the blood is seen in the *falling Hb*, which has been found considerably reduced in many of the cases with normal or nearly normal red cells. Mouisset and Tolot, and Labbe find that the combination of a low Hb-index, excess of polynuclear cells, and absence of fever is a diagnostic sign seldom lacking in early gastric carcinoma, and fails only in some very early cases or when there is some sudden loss of red cells. Yet none of these rules is at all constant.

2. *In the majority of cases of gastric cancer the red cells are reduced in number, the Hb is deficient, and the blood shows the changes of secondary chlorotic anemia.* The reduction of cells is sometimes remarkably slight, but the Hb usually suffers first and most severely. When distinct cachexia is present, both red cells and Hb are nearly

always reduced, unless there is active vomiting. Poikilocytosis appears in the more chronic and severe cases. Nucleated red cells are usually seen in small numbers. Sometimes they are present when the anemia is very slight. Jez found them so common in cancer and so rare in ulcer as to furnish diagnostic evidence between these conditions.

The *Hb-index* is commonly subnormal, but not so low as in chlorosis. Osler and McCrae, however, found an index of 0.63 in fifty-two cases, which is nearly as low as that of chlorosis. In Price-Jones' cases the Hb-index was either high or low, but did not vary much during considerable periods of observation. The Hb is very much less likely to increase when once low, while it does increase, frequently and rapidly, in simple ulcer (Blindeman).

The conditions which tend to develop severe anemia in gastric cancer are chiefly hemorrhage, ulceration, and metastasis. Diffuse growths and annular pyloric tumors have less effect upon the blood.

3. *A moderate proportion of cancers of the stomach pursue a course which clearly resembles that of pernicious anemia.* Without very marked gastric symptoms and with no demonstrable tumor the blood becomes excessively anemic, the red cells falling to a low figure, usually about 1,500,000 to 1,000,000, megalocytes are abundant, and nucleated red cells, some of which may be of large size, make their appearance.

The type of carcinoma found in these cases varies. Sometimes there is a small tumor of the stomach wall with moderate neighboring metastasis, suggesting the development of cancer in an old round ulcer. Sailer, Sadler, and Menetrier report such cases, and the writer has seen two others at autopsy. Usually the tumor is of considerable size and ulcerating, as in another case reported by the writer. It is not probable, however, that the grave form of anemia is necessarily associated with any particular forms of cancer, as both the above types may be seen without extreme anemia. Menetrier and Aubertin discuss the possibility that in these cases the tumor may develop unusually active hemolytic agents.

In the diagnosis of this type of gastric cancer from pernicious anemia one is usually aided in the examination of the blood by the following features:

(*a*) The majority of megalocytes do not show an excess of Hb, which is distinctly more deficient than in pernicious anemia.

(*b*) Megaloblasts are rare, and nucleated red cells, if present, are mostly of normal size or but slightly larger than normal.

(*c*) There is usually polynuclear leucocytosis in advanced carcinoma of stomach.

The leucocytosis may fail and a few megaloblasts may be present, but the writer has been unable to find a case of grave anemia in cancer of the stomach in which the general deficiency of Hb was not marked.

Henry calls attention to the fact that in cancer the cachexia exceeds the oligocythemia, while in pernicious anemia the opposite relation holds, but the anemic type of gastric cancer has in several reported cases reduced the red cells below 1,500,000, the limit noted in his experience. In his statement that at death pernicious anemia leaves fewer red cells (usually below 1,000,000) than gastric cancer (usually above 1,000,000) most observers will concur.

Leucocytosis in Gastric Cancer. Polynuclear leucocytosis is present in the majority of cases, but not in all. In the early stages of the disease, when diagnoses are difficult, leucocytosis is frequently absent, and if we exclude the slight increases the majority of early cases fail to show leucocytosis. This fact probably depends on the usual absence of any cause of leucocytosis which the tumor itself does not furnish. Without much regard to the position of the tumor or the rapidity of growth, leucocytosis makes its appearance as a result of ulceration, exudative inflammation about the tumor, hemorrhage, metastasis, and anemia. In the writer's experience these causes are most active in the order named. A single examination of the blood is, however, quite insufficient to exclude the occurrence of the leucocytosis which is now under consideration. The leucocytosis of secondary anemia is often intermittent, according to the process which it follows, and its detection may require repeated examinations.

The grade of leucocytosis is usually not high, but since in low states of nutrition the leucocytes are usually low, the presence of 10,000 leucocytes has here more significance than in states of health. The majority of advanced cases show between 10,000 and 20,000 white cells. Extreme leucocytosis has usually been found to result from complicating inflammation, extensive ulceration, or numerous metastases. It is said that some cases may pursue their course entirely without leucocytosis.

When the total numbers are distinctly increased the proportion of polynuclear leucocytes is usually high. This fact is especially apparent in the cases reported by Osler and McCrae. Eosinophile cells, however, are nearly always present, and sometimes their numbers and proportions are considerably increased (5 to 6 per cent.). It is of interest to note that Labbe has found many eosinophile cells in the tissue in and about an excised cancer of the stomach as well as in the adjoining lymph nodes.

Digestion Leucocytosis in Cancer of Stomach. R. Müller, in 1890, first called attention to the absence of digestion leucocytosis in cancer of the stomach, and this fact has since been abundantly verified by the reports of Schneyer, Hoffman, Hartung, Capps, Sailer, Cabot, Hassman, and Jez.

Müller, Schneyer, Jez, and Hartung found no digestion leucocytosis in their 41 cases, while in simple ulcer it was always present, except in one case of hematemesis. Hartung found it present in four cases of cancer of other organs. Capps and Cabot report digestion leucocytosis of 3270 to 3850 cells in 3 of 37 cases, while it was present in only 5 of 10 cases of chronic gastritis, and in one case of fibrous stricture of pylorus. More recently Hoffman, finding digestion leucocytosis in 3 of 24 cases of cancer, but in only 2 of 9 cases of ulcer of stomach, and in only 2 of 11 cases of anacidity from other causes, denies any great diagnostic value in the test. Hassman and Schneyer also found no digestion leucocytosis in three cases of ulcer with pyloric stenosis, while Sailer found no digestion leucocytosis in one case of tuberculous cachexia, and in two cases with pre-existing inflammatory leucocytosis. Osler and McCrea demonstrated an increase of 33 per cent. of leucocytes following digestion in 10 of 22 cases of gastric cancer.

The above studies showing that digestion leucocytosis is absent when the stomach fails either to digest or absorb with considerable activity, conditions which usually but not necessarily accompany carcinoma, but which may also be found in simple ulcer, chronic gastritis, stenosis of pylorus, gastroptosis, anacidity, tuberculosis, etc., it appears that this test can hold only a very subordinate position in the diagnosis of cancer of the stomach.

Duodenal ulcer closely resembles gastric ulcer in its effects upon the blood.

Diseases of the Intestine.

The blood is very closely dependent upon the absorptive and excretory functions of the intestine both for its transient variations in concentration and its more permanent supply of albumins. So marked and prompt is the response of the blood to these changing conditions that no examination of the blood requiring accurate calculation should be made without full regard to the condition of the gastro-intestinal tract. The writer has repeatedly seen an attack of typhoid fever completely obliterate the signs of a moderate chlorosis; and has previously referred to the remarkable transformation of the blood observed when, at Montauk, in 1898, typhoid fever developed in subjects of malarial cachexia. Of the two main intestinal functions the blood responds much more promptly to excessive excretion than to increased absorption.

Effects of Absorption. During the early stages of digestion of solid food the red cells tend to increase, owing to the discharge of a considerable bulk of digestive fluids (Buntzen, Sorenson, Leichtenstern). With largely fluid diet, on the other hand, the absorption of fluids may tend from the first to diminish the red cells, so that their lowest proportion is found about four hours after meals. At the same time coagulation is slower (Vierordt, Leichtenstern). The increased flow of lymph doubtless contributes to this result. With proper diet in healthy adults these processes usually fail to cause important variations in the red cells and Hb of the blood, and even when the quantity of fluid in the food is excessive or extremely deficient the demonstrable effects are slight and very transient. Leichtenstern could detect no change in the blood of a patient who drank 21.5 litres of water during three days, and Schmaltz found only a slight reduction in gravity (1.059 to 1.057) forty-five minutes after the ingestion of 4 litres of physiological salt solution. The ingestion of very large quantities of fluid can dilute the blood only to a slight degree, beyond which the activity of the kidneys is sufficient to carry off all excess and keep the gravity of the blood within somewhat narrow limits.

Depletion of the blood by limited ingestion of fluids and especially by watery exudates *leads, on the other hand, to very prompt and considerable concentration of the blood.* Numerous clinical illustrations of this fact have already been considered as arising from disorders of the stomach, but the more extreme examples are connected with the use of purges and with diseases of the intestine, especially those attended with active diarrhea.

Effect of Purges upon the Blood. It was held by Poisseuil in 1839 that when salines are administered in greater concentration than that of the blood serum, the laws of osmosis determine the passage of fluids from the blood into the intestine. It has since been shown that with many laxatives other factors lead to the same result, especially the increased intestinal secretion and the failure of absorption.

Brouardel first demonstrated an increase of blood cells following purgation, finding that with severe diarrhea the red cells might rise as much as 1,456,000 per c.mm. The leucocytes were at the same time increased, maximum 5880, but apparently for different reasons and not in proportion to the red cells. Later, Hay found an increase of nearly 2,000,000 red cells within one and a half hours after the administration of a large dose of Glauber's salt. The increase began in a few minutes, and the normal condition was restored in about four hours. The effects were more marked the more concentrated the solution; but after a certain concentration of blood had been reached, no further effects could be induced and no purgation followed the continued administration of the saline. Likewise Grawitz found the administration of 15 grains of Epsom salt in 50 c.c. water to raise the gravity of the blood, beginning in five minutes and reaching a maximum increase, from 1.0591 to 1.0539, in forty-two minutes, after which there was a steady decline. Common salt was even more active, 15 grains in a little water raising the gravity of the blood from 1.050 to 1.060 within twenty minutes. The effects of such salines he found were largely nullified when the stomach and intestines contained much food.

Colitis (Dysentery). **Acute colitis** of croupous or ulcerative type usually causes some concentration of the blood and is attended with considerable leucocytosis. After the acute febrile period, if the patient survives, anemia replaces the oligemia and the leucocytosis becomes intermittent. Yet the writer has seen excessively emaciated cases of acute amebic colitis die, without fever, and without marked reduction in the proportion of red cells.

Chronic ulcerative colitis, when attended by much purulent exudate and numerous hemorrhages, may lead to extreme anemia of secondary chlorotic or pernicious type. Leucocytosis if present is intermittent.

Catarrhal colitis has little effect upon the blood, tending by depletion to raise the proportion of red cells. There is usually no leucocytosis.

Simple acute diarrhea causes a slight transitory increase of cells and Hb.

Cholera. The most extreme grades of concentration of the blood are seen in those cases of Asiatic cholera which die in the algid stage, and in which the system has been excessively depleted by prolonged diarrhea. The diminution in the quantity of blood may even be plainly visible to the naked eye, in the scanty hemorrhage which follows section of an artery (Dieffenbach).

The principal changes in the blood of cholera are fully set forth in the first systematic study of the subject by Schmidt, who in specimens obtained by venesection found the specific gravity of the whole blood raised from 1.050 or 1.059 to 1.065 or 1.073, of the serum from 1.029 to 1.047. Even the red cells were found to suffer a marked loss of water, their gravity rising from 1.088 to 1.102.

Moreover, while the increase in density of the blood and serum was not uniform nor in all cases well marked, the changes in the red cells were pronounced and invariable. Schmidt's analyses were not made in the most extreme cases, however; and the relation between the gravities of the blood, the cells, and the serum indicates that the relative quantity of serum had remained about normal. Other and older observers (Thomson) had previously noted a gravity of the serum as high as 1.057, indicating that the serum had begun to suffer disproportionately in bulk, and that a true *oligoplasmia* existed.

Schmidt and Biernacki added to the knowledge of the subject the fact that all chemical constituents are increased in rather uniform proportion except the sodium chloride, which seems to transude in the stools in excessive quantity. Biernacki, finally, has shown that these excessive grades of depletion of the blood do not exist unless the tissues are themselves seriously drained of fluids and can no longer supply water to the blood.

The **coagulability** was found by Hayem and Winter to be sometimes normal, but often, in the stage of reaction, much increased. They found a much diminished capacity of the blood to absorb oxygen.

The **alkalescence** of the blood in cholera was regarded by the older observers as extremely deficient, and Cantani in 1884 claimed to have demonstrated a rapid decrease of alkalinity, a neutral reaction during the algid stage, and even an acid reaction before death. This change he referred to excess of CO_2, and he recommended treatment by alkalies. Biernacki refers the diminished alkalinity to loss of sodium. Hayem and Winter examined twelve specimens of blood during the stage of collapse, finding four of them neutral or slightly alkaline, and eight slightly acid. The reaction has not been determined by more recent methods.

The **red cells** are markedly increased in nearly all cases. In one instance they rose to 7,662,000 within twenty-four hours, the patient finally recovering. Usually the red cells rise to 6,500,000 or 7,500,000, and this excess is usually but not always proportionate to the other evidences of concentration. One case showed a maximum of over 8,000,000 red cells, while another gave only 3,193,000 during the stage of reaction. Beginning concentration has been noted as early as the third hour. Biernacki attributes to the thickening of the blood and especially to the depletion of tissues considerable importance in the pathogenesis of symptoms, but found very severe symptoms in patients not showing much diarrhea. Rogers found uniform polycythemia reaching over 8,000,000 in two fatal cases, but could not detect any relation between the concentration of the blood and the ultimate course of the disease.

Morphological changes in the red cells have not been noted by most observers, but Nicati reports that in hemorrhagic and jaundiced cases there are many shadows of dissolving red cells, and often pigmented leucocytes, to be seen, especially in the stage of reaction.

Leucocytosis is practically constant in all marked cases. Biernacki found 21,250 cells in one mild case suffering chiefly from diarrhea, but only 4375 in another very similar case. Eight cases showing in the algid stage very high leucocytosis, 40,000 to 60,000, were fatal. Some cases with more moderate leucocytosis (20,000 to 30,000) were also fatal, but less rapidly. In the stage of reaction the leucocytes are usually lower, but some fatal cases show a persistent or increasing leucocytosis in this stage. The increase of leucocytes is relatively much greater than that of the red cells. It has been noted as early as the twelfth hour, and may persist as long as six days. The polynuclear cells are in great excess, and eosinophiles are usually absent. Rogers observed leucocytosis in proportion to the severity of the disease. He describes as characteristic an increase in the number and proportion of large mononuclear cells, especially in fatal cases. When these cells exceeded 10 per cent., the patient usually died.

Bacteriology of the Blood. The *comma bacillus* has apparently not been found in the blood during life. After death it was recovered from the heart's blood in a small proportion of forty-eight cases examined by Lesage and Macaigne, and in four of eleven cases examined eight to twenty-four hours after death by Rokowsky. Wlajew failed to obtain positive results from the heart's blood in several cases, and one specimen of blood, drawn during life, was sterile and non-pathogenic to animals. Diatroptoff also found three sterile specimens of heart's blood.

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CHAPTER XXI.

LUNGS, HEART, KIDNEYS.

DISEASES OF THE LUNGS.

Respiratory Changes in the Blood. During its passage through the lungs the blood undergoes certain changes which in their normal progress belong to the physiology of the blood, but when disturbed by pathological conditions may considerably affect both its chemistry and its morphology.

The absorption of O_2, which results partly by simple absorption by the plasma, but largely by chemical union with Hb, raises the volume of this gas in arterial blood to 21 per cent., as aganist 12 per cent. for venous blood, while the discharge of CO_2 from the tissues into the plasma, where about two-thirds of it exist as a bicarbonate and one-third in the red cells, increases the CO_2 content of venous blood to 46 volumes per cent. from 38 per cent., the average content of arterial blood.

The character of the diet considerably influences the ratio of CO_2 excretion to O_2 absorption, which with carbohydrate food maintains a proportion of about 1 to 1, while with highly albuminous diet more oxygen is absorbed and devoted to oxidation, and the ratio is 0.74 to 1 (Pembry).

Arterial blood was long supposed to be richer than venous in solids and Hb (Kruger), but Cohnstein and Zuntz showed this apparent thickening to be due to stasis caused by the procedures followed in venesection ; and with Kruger and others these observers demonstrated that the blood in larger arteries and veins is equally rich in red cells, Hb, and dry residue.

An important difference between venous and arterial blood was demonstrated by Hamburger, who showed that under the influence of CO_2 the isotonic tension of the red cells is moderately increased, so that chlorides and water pass more readily from plasma into cells, while albumins, alkalies, and probably also phosphoric acid, pass from cells to plasma. A simultaneous increase of the sugar and fat of the serum was also noted, but its origin not fully determined.

As a result of these alterations in osmotic characters, the red cells of venous blood were found to swell and assume a more spheroidal shape. Limbeck, who also verified this change in the osmotic qualities of the red cells in venous blood, referred the excess of dry residue of the plasma not to withdrawal of albumins from the cells, but to absorption of the water of the plasma by the red cells, which he found to suffer an increase of volume in venous blood.

With the increase of CO_2 in venous blood, those observers who identify alkalinity with CO_2-content found an increased acid-neutralizing power in venous blood. This anomalous result, viz., the increase of alkalinity of a fluid by the addition of an acid, they referred to the withdrawal of alkaline salts from the tissues for the neutralization of the acid.

It is of prime importance, in considering the pathogenesis of symptoms in various anemias, to call attention to several studies which have demonstrated that Hb has a strong chemical affinity for oxygen, and that this attraction, rather than the percentage of O_2 in the air, chiefly regulates the supply of oxygen to the tissues. Lukjanow first showed that by increasing the pressure of the O_2 in the lungs he could not add to the quantity of absorbed oxygen, although arterial blood is not saturated with oxygen, while Lowy found that neither by increasing the pressure to 1400 mg. Hg nor by lowering it to 40 mg. (normal 760 mg.) could the blood be made to absorb more or less O_2. Beyond these points, however, the absorption of O_2 begins to vary. Turning their attention to the respiratory processes in anemia, Kraus, Chvostek, Bohland, and especially Biernacki, found that the quantity of oxygen that can be exhausted from blood remains about the same, although the Hb may vary greatly, that *in simple anemia the system instead of using less oxygen employs rather more than in health, and only in the severest forms of anemia is the percentage of oxygen in the blood distinctly reduced.*

It therefore appears, as Grawitz concludes, that the oxygenation of the blood in the lungs is not regulated by the laws of diffusion, and is not closely dependent upon the atmospheric pressure, nor upon the amount of Hb in the blood, but is governed by a special chemical activity residing in the Hb.

The laws governing the respiratory changes in the blood, thus briefly sketched, principally apply to chemical processes, but have important bearing, also, upon certain morphological changes seen in conditions of dyspnea, asphyxia, and in the peculiar state of the blood observed at high altitudes.

The exhalation of vapor during respiration, while not followed under normal conditions (Dastre) by any concentration of the blood, may, if we except the results of Grawitz's observations in pathological states, lead to considerable loss of water and increase of red cells and dry residue. Grawitz examined the blood of an hysterical woman, before and after a prolonged attack of hysterical dyspnea, in which the respirations rose to 100 per minute, and found the red cells increased from 4,900,000 to 5,110,000, the dry residue of the whole blood from 19.78 per cent. to 22.08 per cent., and that of the serum from 9.54 per cent. to 9.85 per cent. A similar result was noted after he had voluntarily increased his own respiration to 40 per minute, during a period of one hour and three-fourths.

Asphyxia. During dyspnea from disease of heart or lungs there is a proportionate increase of CO_2 in the blood (Kraus, Chvostek). In extreme asphyxia, Zuntz collected the results of nineteen analyses, which gave for O_2 0.96 volume per cent. and 19.53 per cent. of CO_2.

In the attempt to discharge CO_2 from the tissues and to supply O_2, respiration is accelerated, and the same result is attained by the polycythemia which results from venous stasis. Among the results following the excess of CO_2 in the blood is an alteration in the red cells which renders the Hb more readily diffusible in the plasma. This change, while not apparently leading to hemoglobinemia during life, is evident after death in the reddish tinge of the serum. One of the prominent signs in the blood of asphyxia is the failure of coagulation, which is often very complete and is probably referable to the presence of CO_2. Ottolenghi, examining the blood of asphyxiated rabbits, found it to be thinner and more diffusible, while the red cells were reduced in number, resistance, and specific gravity. In the peripheral blood of the human subject, during rapid asphyxia, there are all the evidences of extreme concentration. In the slower forms of asphyxia, which are accompanied by pallor, the blood has apparently not been compared with that of the more rapid cases.

Emphysema. The blood in emphysema varies considerably according to the state of the circulation and respiration.

During the intervals when the circulation is at its best, and cyanosis is absent, an anemic condition may be demonstrated in many cases, which is referable to the malnutrition of the patients or to complications, especially cirrhosis of liver, nephritis, and chronic gastritis. *When cyanosis becomes chronic*, most observers have found more or less polycythemia and increase in specific gravity (Peiper,[1] Grawitz[2]), but in Leichtenstern's cases this concentration was apparently insufficient to obscure the anemia, as he reported a loss of Hb in patients suffering from cyanosis, edema, and heart-failure. An increase in the volume of red cells up to 8μ has been described by Vaquez,[1] in cases of chronic cyanosis from various causes. *During asthmatic attacks* with cyanosis, the blood becomes still further concentrated, and the expressed drop may appear almost black.

The leucocytes are usually of high normal average, but during the asthmatic attacks there may be distinct leucocytosis. When bronchitis is added the leucocytes may be markedly increased. With or without leucocytosis a considerable proportion of the leucocytes may be of the eosinophile variety. Gabritschewsky, who called attention to this *excess of eosinophiles* in the blood corresponding to their increase in the sputum of many asthmatic patients, found in three cases from 11 to 22 per cent., while Fisk reported 14.6 per cent. in one case, and Billings found as many as 53.6 per cent. out of 8300 cells. From the observations of Leyden, Van Noorden, and especially of Schwerschewski, it has been shown that excess of eosinophiles in the blood of asthmatics occurs principally or only at the time of the paroxysm, that they increase with repeated attacks, diminish shortly after the paroxysm and with the appearance of expectoration, and in the intervals largely disappear. Since there is no such tendency to eosinophilia in the suffocative attacks of endocarditis, nor in the severe dyspnea of Bright's disease, the examination of the blood may serve to distinguish bronchial asthma from other forms of dyspnea, and may perhaps indicate the approach of a paroxysm.

Teichmüller and Fuchs, however, find many eosinophile cells in other forms of bronchitis.

Bronchitis. **Acute catarrhal bronchitis** of ordinary severity and affecting the larger bronchi only, has little effect upon the red cells, and seldom raises the number of leucocytes. In some severe cases the writer has found as many as 15,000 white cells, when the patient's temperature was 104°. In some of Cabot's cases the leucocytosis was slightly higher. In seven children between three and seven years of age, Stengel and White observed moderate polynuclear leucocytosis, 13,000 to 19,000 cells.

Capillary bronchitis, with high temperature and marked prostration, may strongly resemble lobar pneumonia in clinical features, and, like pneumonia, is accompanied by marked leucocytosis. In three fatal cases with high temperature, dyspnea, and cyanosis, the writer found 18,000 to 37,000 leucocytes. At autopsy there were signs of severe general bronchitis, no consolidation, but well-marked chronic nephritis. In two cases without autopsy, Cabot found as high as 41,000 leucocytes.

DISEASES OF THE HEART.

The majority of observations on the blood of chronic endocarditis have shown more or less continuous polycythemia and concentration of the blood. The more important of these studies have been contributed by Nasse, Naunyn, Toenissen, Bamberger, Lichtheim, and Reinert,[2] who have referred this concentration to stasis from local vasomotor or general cardiac paresis, to transudation of serum, or (Naunyn) to a physiological effort on nature's part to furnish more oxygen by increasing the cells and Hb of the blood.

The opposite condition of hydremia has been found more usual or regarded as more important by Leichtenstern, Oertel, Oppenheimer, Stintzing and Gumprecht, and many others, who hold that an excess of lymph from various sources passes into the vessels, as a result of lowered blood pressure. Schneider and Limbeck find that *while mitral lesions are usually associated with polycythemia, aortic insufficiency more often leads to hydremia.*

All observers are agreed that the valvular lesion, and the usual causes leading to it, exert little or no effect upon the blood, and, while differing as to their significance, all admit that the changes in the blood vary considerably at different periods of the disease.

The conflicting views have been carefully studied and partially unified by Grawitz, who finds that the blood shows three different states, according to the stage of the disease, and determined chiefly by the degree of compensation established in the heart:

1. When valvular lesions are fully compensated by cardiac hypertrophy, and when there are few or no symptoms of disease of this or other organs, the state of the blood depends entirely on the constitution and state of nutrition of the individual.

2. When disturbances of compensation begin, when the heart muscle fails and the pulse is accelerated, and dyspnea and its train of

symptoms appear, changes occur in the blood which are clearly referable to the diminished force of the heart.

The whole blood loses in specific gravity and in dry residue, while its content of water increases, and these changes are more marked in the veins than in the superficial capillaries. The red cells are reduced in number, but they suffer no changes in size or in Hb-content. The leucocytes show no characteristic changes. The blood serum shows the most marked changes, always becoming more watery. These alterations Grawitz refers solely to the lowered blood pressure, which is followed by dilatation of capillaries and passage of tissue fluids into the blood stream. Oliguria may result from the same factors, but is not the cause of the hydremia.

3. When chronic venous stasis is established, and dyspnea, cyanosis, and edema exist, the changes in the blood are more complicated.

Under these circumstances the blood loses water, becomes richer in red cells, and more so in the capillaries than in the veins. These effects Grawitz believes to result from transudation of fluids through the capillaries into the tissues, but increased exhalation from the congested lungs, and possibly also increased evaporation from the skin, contribute to the same result. When the balance of pressure between capillaries and tissues has been adjusted, any added failure of the heart may be followed by still lower pressure and temporary hydremia.

Grawitz's deductions have appeared to most critics to be only partially valid. In the first stage it is generally agreed that the blood is usually normal, and it is also fully demonstrated that a fall of blood pressure leads to relative hydremia, but his explanation of the origin of the polycythemia of chronic endocarditis has not proven fully satisfactory. As Limbeck points out, many patients always show some reduction of red cells whether compensation is complete or not; while others show considerable variations in red cells without apparent relation to the action of the heart, the presence or absence of edema, or the activity of the kidneys. Limbeck, therefore, attributes less importance to the interchange of fluids between the blood and tissues, and believes that the polycythemia of heart disease is partly referable to the same obscure factors which increase the red cells in high altitudes. That there is an actual new formation of red cells in the polycythemia of endocarditis is held by Marie, Reinert,[2] and others.

Without entering further into the details of individual opinions, it appears certain from the studies already cited, and from others contributed by Siegl, Peiper,[1] Schmaltz, Banholzer, Maxon, Stintzing and Gumprecht, that:

1. In advanced endocarditis with failing compensation there is a distinct tendency toward concentration of the blood, which at times increases the red cells to 8,000,000 or more.

2. This polycythemia is the combined result of venous stasis, dyspnea and cyanosis, transudation of blood serum, and possibly also of other obscure factors which lead to polycythemia in high altitudes.

3. The state of the blood in chronic endocarditis responds to a limited extent, but not invariably, to diuretics, diaphoretics, purges, and heart tonics.

4. The constant tendency toward anemia is usually masked by the peculiar condition of the circulation.

5. The result of the examination of the blood must be interpreted with strict regard to the general condition of the patient.

Results of Blood Examinations in Chronic Endocarditis. The foregoing statements are fully borne out by a review of the reported examinations of the blood in endocarditis. In some series of cases the polycythemia is very uniform, as found especially by Oppenheimer and Reinert, while the Hb commonly runs from 90 to 110 per cent. Usually the red cells do not exceed 6,000,000, but some cases with cyanosis reach 7,000,000 or even 8,000,000. On the other hand, a slight reduction of cells, not below 4,000,000, appears in most cases reported by other observers. Maxon's statement that the blood of heart disease commonly shows slight variations above or below the normal in red cells, specific gravity, and albumins, is the only rule of general application.

That aortic insufficiency is usually found with less concentrated blood is also apparent in nearly all comparative studies (Schneider, Grawitz, Limbeck, Menicanti, Reinert, Hayem, Sadler). Yet even here the red cells seldom fall below 4,000,000.

Variations from the above rules are not unknown, as some cases with cyanosis have shown less than 4,000,000 red cells, and the aortic insufficiency may lead to polycythemia. Much depends upon the particular circumstances surrounding each patient, which must always be regarded with care

The LEUCOCYTES in uncomplicated cases show no important variations from the normal, but with concentrated blood they are apt to be rather above the average normal figures. During the febrile periods which mark the terminal stages of many cases, leucocytosis of considerable grade is usual. The complications of endocarditis which cause leucocytosis are numerous, and antemortem leucocytosis is usually prolonged and pronounced.

Malignant Endocarditis. The more acute cases of bacterial endocarditis furnish some of the most typical examples of septicemia, in which the blood shows rapid loss of cells, albumins, and Hb, leucocytosis, and very often bacteria in demonstrable numbers.

The anemia of malignant endocarditis is of rapid progress and usually becomes one of the characteristic features of the disease. Beginning in patients with practically normal blood, the septic process often reduces the red cells within two to three weeks to 3,500,000 or 3,000,000, or even lower. In some cases, usually those of longer duration, the anemia is less marked (over 4,000,000 cells), or is masked by the febrile process and by venous stasis. In all the markedly septic cases, however, the anemia becomes severe. In the later stages of severe cases the evidences of destruction of red cells become marked, the Hb is very deficient and may become dissolved in the plasma, shadow corpuscles appear in the fresh specimen, and various forms of degeneration of red cells are demonstrable in the dry specimen. In such cases the red cells may not number much over 1,000,000. After death the deposits of blood pigment in the viscera are very abundant and closely simulate those of pernicious malaria, for which they may readily be mistaken.

Leucocytosis is present probably in all cases, but is often intermittent and single observations may fail to discover it. In this, as in other forms of sepsis, there may be a tendency to slight leucocytosis with high proportion of polynuclear cells, and in general the increase of white cells is not very marked, considering the condition of the patient. Roscher and Cabot report fatal cases with 8000 and 8900 leucocytes shortly before death, while Krebs found antemortem leucocytosis of 44,200, and Grawitz reported 168,000.

Bacteriological examination of the blood is often required in the diagnosis of obscure cases. From the rather limited number of recorded examinations by reliable methods it appears that bacteria are always to be found in the blood in a certain group of cases of ulcerative endocarditis. These cases include the examples of cryptogenic infection which run an acute septic course and end fatally within a few weeks or months. In the cases of ulcerative endocarditis which terminate with septic fever, and in which an acute process is often added to the chronic lesion, cultures of the blood are usually negative. Grawitz's claim that repeated negative cultures are sufficient to rule out ulcerative endocarditis, cannot, however, be admitted ; for the evidence rather favors Kuhnau's view that the difference between "simple" ulcerative and malignant endocarditis is one of degree, not of kind, and that in many cases of subacute or chronic ulcerative endocarditis of bacterial origin the blood is sterile.

These groups of cases are illustrated by the reports of several investigators. Grawitz[2] examined 7 cases of suspected ulcerative endocarditis, all of which gave negative cultures, but only 1 of which proved to have an ulcerative lesion. In 3 pronounced cases of malignant endocarditis he obtained in 2 *staphylococcus pyogenes aureus*, in 1 *diplococcus lanceolatus*. Kraus examined the blood in 7 cases, finding the streptococcus in 1, negative cultures in 6. Petruschky, examining the blood from a wet cup, obtained the *streptococcus pyogenes* in 1, and a negative result in the other, of 2 cases. Kuhnau had only 1 positive result (*staphylococcus pyogenes aureus*) in 12 cases examined. Cohn in 2 acute cases found in 1 both *streptococcus pyogenes* and *staphylococcus aureus*, and in the other *staphylococcus aureus* alone, while in 2 chronic cases the cultures were negative. James and Tuttle obtained positive cultures in 3 of 4 fatal cases, once finding the *diplococcus lanceolatus*. White obtained negative results in 2 cases three to four days before death, but isolated *staphylococcus pyogenes aureus* from both just before death. The writer, using 5 to 10 c.c. of blood drawn from the median basilic vein, obtained pure cultures of *streptococcus pyogenes* in 2 typical cases of malignant endocarditis. These patients gave no history of cardiac disease until the development of a septic febrile process, which continued without intermission for one to six months, and until death. In 4 cases of chronic ulcerative endocarditis ending with septic fever cultures of the blood were negative.

Gonorrheal Endocarditis. The *gonococcus* has been obtained in pure culture from the circulating blood of cases of endocarditis by Thayer and Blumer, and Thayer and Lazear, and Halle. In all cases the blood was extremely anemic, red cells under 2,000,000, while the leucocytosis was slight, 12,000 to 14,000 in one case, and 8500 to 18,000 in the others. Many cases of gonorrheal septicemia collected by these authors indicate the frequency with which this germ is probably connected with endocardial lesions.

Columbini also obtained the gonococcus in cultures of the circulating blood in a case of gonorrheal septicemia with endocarditis, and demonstrated its identity and pathogenic qualities in the human urethra.

McCallum and Hastings report a case of acute endocarditis from which they obtained from the circulating blood cultures of a somewhat peculiar coccus resembling *streptococcus pyogenes.*

Significance of the Bacteriological Examination of the Blood in Endocarditis. 1. A positive result when obtained under proper precautions, and long enough before death to avoid antemortem secondary infections, places beyond doubt the infectious nature of the process.

2. A negative result does not indicate that the vegetations do not contain bacteria in their substance or on their surface. A large number of negative bacteriological examinations of the blood have been recorded in cases showing, *postmortem*, various bacteria in and on the inflamed heart valves.

From personal experience of moderate extent the writer has drawn the impression that when malignant endocarditis follows the type of pure septicemia with cardiac symptoms in the background, bacteriological examination of the blood is usually positive, but when cardiac symptoms are or have been prominent, bacteriological examination of the blood is usually negative.

Congenital Heart Disease. The pronounced cyanosis from which most of these patients suffer leads to extreme degrees of concentration of the blood, as the following cases will show:

Author.	Red cells.	Leucocytes.	Hb%.	Specific gravity.
Krehl	8,000,000			
Vaquez[2]	8,900,000			
Banholzer	9,440,000	....	160	1.071
	8,470,000	12,000	110	
Gibson	6,700,000	12,000	92	
Carmichael	8,100,000	16,000		
	8,820,000			
Toenissen	7,540,000			
Cabot	8,430,000			

Townsend has recently reported thirteen cases in which the red cells varied between 5,600,000 and 11,800,000.

DISEASES OF THE KIDNEY.

The existence of marked hydremia in cases of nephritis was demonstrated in several earlier studies of the chemistry of the blood (cited by Gorup-Besanez), but Schmidt's analyses first clearly showed the more exact nature of this hydremia.

In three patients with albuminous urine and marked edema, he found considerable loss in specific gravity of the whole blood (1.043 to 1.051), and of the serum (1.018 to 1.024), while the red cells remained nearly normal in gravity (1.081 to 1.084), but were much reduced in bulk (34.2 from 44.9 per cent.). These changes were believed to result largely from the loss of albumins of the serum, while the Hb of the red cells and the salts of the plasma were but slightly affected. Scherer's analyses in six cases demonstrated a decrease in the solids of the whole blood, and a loss of 5 per cent. of the albumins, although the fibrin was sometimes increased. The red cells were reduced, but their dry residue relatively increased. Although the serum was very watery, the dry residue averaging 6.9 per cent., the salts of the serum were increased. Very low gravity of the serum, 1.019 to 1.023, was also noted by Becquerel and Rodier.

From numerous other analyses by Frerichs, Gorup-Besanez, Hinterberger, and others, it began to appear that *the composition of the blood varies considerably in different stages of the disease*, and later studies have shown that the two main clinical and pathological types of the disease, the chronic exudative and the chronic productive without exudation, are attended with distinct changes in the blood.

Chronic Exudative (Parenchymatous) Nephritis. The albuminuria and edema of this group of cases are associated with well-marked anemia, which, however, is subject to great variations on account of the intermittent course of the disease and of the frequent disturbing effects of treatment.

The usual condition of the blood is one of moderate chlorotic anemia. The majority of cases show between 3,000,000 and 5,000,000 red cells and 40 to 80 per cent. of Hb. The reported series of cases in the literature show wide variations in the condition of the blood. Considerable polycythemia is not infrequently encountered. Leichtenstern and Sorensen found little reduction of Hb and an average of 4,740,000 red cells, while Laache and Reinert[1] found a low Hb percentage with slight loss of cells. These changes represent the condition most commonly found. Cases with less than 2,000,000 cells are reported by Grawitz, Sadler, Cabot, and are not rare. In these and occasionally in other cases the writer has sometimes found a high or increased Hb-index, and at autopsies in cases of pernicious anemia has several times found the lesions of chronic productive nephritis with exudation.

These variations are well illustrated by Cabot's synopsis of thirty-five cases from the Massachusetts General Hospital :

Red cells.	Cases.	Red cells.	Cases.
6,000,000 to 7,000,000 . . .	3	3,000,000 to 4,000,000 . . .	11
5,000,000 to 6,000,000 . . .	6	2,000,000 to 3,000,000 . . .	2
4,000,000 to 5,000,000 . . .	12	1,000,000 to 2,000,000 . . .	1

The attempt to analyze the causes of the variations of the anemia in chronic nephritis is met with difficulties, and numerous factors must be considered. Polycythemia results from cyanosis, disturbance of circulation, and rapid transudations, and each of these conditions may and frequently does obscure pronounced anemia. An astonishing degree of pallor of the face may thus be found with 5,000,000 red cells and 100 per cent. of Hb. The progressive anemia of the average case is clearly referable to the loss of albumins of the serum and general malnutrition. Yet the real advance of the anemia is often disturbed by the intermittent losses of albumin and attacks of edema. While Benczur and Czatary, and v. Jaksch[1] believe that the hydremia is not proportionate to the edema, yet Bogdanow, and Stintzing and Gumprecht have shown a positive connection between hydremia and edema, and have noted an improvement in the blood following improvement in general symptoms.

The relatively high Hb-index is apparently the natural result of a loss of albumin which affects principally the serum. The gravity of the red cells has been found uniformly high by most analysts since the time of Scherer.

Grave or pernicious anemia develops in a small proportion of cases of chronic parenchymatous nephritis, and appears at times to result directly from nephritis. It must be referred to the repeated losses of albumin from the blood and to the general disturbance of nutrition, but it is probable that the very severe grades of anemia result from the combined effects of lesions in several viscera, including chronic gastritis, cirrhosis of liver, arteriosclerosis, etc.

Chemistry. Chemical analysis gives the clearest insight into the changes in the blood in nephritis. The specific gravity is regularly reduced. Peiper[1] found a gravity of 1.026 in a very anemic child, but after marked improvement four weeks later the gravity was 1.055. Similar variations, usually between these limits, are reported by various observers. The changes in the serum demonstrated by the older observers have been verified by many later studies. The gravity of the serum is much reduced (as low as 1.013, Bostock), and its volume remains high. While considerable relation has been found to exist between the changes in the blood and the albuminuria and edema (Stintzing, Gumprecht, Bogdanow), the immediate effects of transudates and albuminuria are not always evident (v. Jaksch). Hammarschlag, who found the gravity of the serum between 1.018 and 1.030, concluded that *edema has more effect upon the gravity of the serum than has albuminuria ; and that when edema is absent the gravity of the serum is usually about normal.*

The *leucocytes* in chronic nephritis are usually normal or subnormal in number. Cabot found no leucocytosis in thirty-one of forty unclassified cases, while in fourteen of nineteen uremic cases there was leucocytosis, reaching 44,000 in one eclamptic patient. Other reports show similar variations, and it is evident that the behavior of the leucocytes in chronic nephritis depends upon accidental conditions and complications.

Chronic Interstitial Nephritis. (**Contracted Kidney.**) The absence of edema and marked albuminuria in cases of chronic nephritis without exudation allows the blood to remain practically normal, at least during the latent progress of the disease. Many patients are carried off by acute uremia and other terminations of interstitial nephritis before the blood is markedly altered. If at any time in the progress of the disease there are exacerbations marked by exudation, albuminuria, and edema the blood suffers for a time as in chronic nephritis of more distinctly exudative type. After the exudative period the blood is partially restored, but a certain grade of chlorotic anemia is apt to persist.

That chronic nephritis may run its course without giving notable changes in the blood, or, indeed, any other pronounced symptoms, is a well-attested fact, of which the writer has seen at autopsy at least one instance. This subject was an able-bodied policeman, dying of acute colitis, whose kidneys were shrunken to an extreme degree. The blood and urine were normal shortly before death.

On the other hand, very severe grades of anemia, usually of microcytic type, are sometimes associated with chronic diffuse nephritis without exudation. Some of these cases are partly referable to lead poisoning, which has initiated the renal lesion and damaged the blood. In others there is advanced arteriosclerosis with frequent hemorrhages from the nose, kidneys, or other regions. In still others no reasonable explanation of the anemia is to be found.

Grawitz recognizes two stages in this disease: *one* in which the heart and circulation and the blood are normal; and a *second* in which compensation fails in the hypertrophied heart, and the blood suffers the same changes as in uncompensated valvular disease. The importance of the condition of the heart in determining the blood changes in nephritis is very great and has already been considered in the references to edema and cyanosis but failure of the left ventricle with feeble pulse is more common with the large white than with the small contracted kidneys. The writer is, therefore, unable to recognize the above stages in the course of the contracted kidney, finding them more evident in the cases of chronic diffuse nephritis with exudation, and in patients with albuminuria and edema.

Acute Nephritis. The changes in the blood in acute nephritis with albuminuria and edema resemble those of the same type of chronic nephritis. Peiper found no diminution in specific gravity in an acute case. Laache and Bogdanow observed more rapid, severe, and more variable changes in the acute than in the chronic cases, while v. Jaksch concluded that in both forms the changes were about equally variable. Hayem found no considerable loss of red cells except in hemorrhagic cases. Many other isolated reports show all but the very severe grades of anemia developing within a few weeks, sometimes very rapidly.

Leucocytosis (maximum, 22,000) has been observed in a considerable proportion of cases by Hayem, Sadler, and Cabot, the latter author referring the persistent increase to loss of blood by the kidney.

None of these observers has attempted to connect the behavior of the leucocytes with the character of the lesion in the kidneys. It would seem that the productive inflammation could run a fatal course without leucocytosis, that simple acute exudative nephritis might be accompanied by a moderate increase of white cells, and that acute idiopathic or secondary purulent nephritis must nearly always be accompanied by marked leucocytosis. Unfortunately there are no reports at hand on which to base these conclusions. Profuse hemorrhage, uremic attacks, and complicating infections may be responsible for occasional leucocytosis, but Sadler's negative cases show that much albumin, many granular casts, and blood cells may be present in the urine when the leucocytes in the blood are not increased.

Uremia. **Chemistry.** Very numerous chemical analyses of the blood in uremia have failed as yet to demonstrate the true nature of this intoxication. UREA in the blood was believed by Frerichs to be the particular toxic agent in uremia, and although this principle has been demonstrated in abnormal quantities in the blood of uremic coma by Spiegelberg, Hoppe-Seyler, Bartels, and others, it was shown by Landois that intravenous injections or local applications to the medulla of large quantities of urea exert no toxic influence. Neither has its derivative, ammonium carbonate, been found in the blood, nor shown to exert a toxic influence on the nervous system

(Kuhne, Strauch). **Kreatinin**, believed by Schottin and Perls to be the active agent, has likewise been set aside, although this substance is increased in the blood of uremic subjects. **Potassium** has been found in considerable excess in the blood by Feltz and Ritter, and by Astaschewsky, who regard uremia as a form of potassium poisoning. Although Sneyers and Horbaczewsky failed to find an excess of potash salts in eclamptic and uremic subjects, this theory has received more recent support from Bouchard, Roger, Rovighi, and others, who offer evidence to show that potassium is one of several poisonous substances which accumulate in the blood in uremia. Limbeck has shown, however, that while an excess of potash can be obtained from the blood of uremic dogs, he could get no such excess if the blood was examined before death.

Diminished alkalescence was first noted in uremic blood by v. Jaksch,[2] and subsequently by Peiper,[2] Rumpf, Mya and Tassinari, Limbeck, and others, who have shown that the alkalescence diminishes greatly on the approach of uremic symptoms. Brandenburg found the alkali-tension greatly diminished in uremia. The attempt to establish this theory of acid intoxication has, however, not been successful. Although uric acid may be in excess in uremia, it is also quite as much increased in many other conditions. Fatty acids and phosphoric acids were not found in excess by Limbeck, who, indeed, was unable to demonstrate a deficiency of CO_2 in all cases of experimental uremia. Diminished alkalescence appears more probably to be only a secondary condition of the blood in uremia.

The *present tendency* is to regard the uremic seizure as the result of toxic action of a variety of nitrogenous metabolic products which are supposed to be retained in the system or at times thrown off in excess in the urine. Bouchard and others have isolated from the urine a variety of toxic principles, including ptomains and urotoxins, some of which produce convulsions; others are narcotic, while still others lower temperature, contract the pupil, or produce salivation. Although the actual existence of these principles has been denied (Stadthagen), and the urine has not always been found so toxic as Bouchard claims it to be (Fleischer), the autotoxic theory is generally regarded as approaching most nearly to the true explanation of uremia. One of the chief objections to it consists in the fact that uremic attacks frequently arise at periods when the excretion of urine is much improved.

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CHAPTER XXII.

MALIGNANT TUMORS.

CARCINOMA.

THE impression obtained by individual observers of the effect of carcinoma upon the blood has largely depended upon the class of cases encountered.

In active hospital wards the cases are usually advanced, cachexia is distinct, and routine examination of the blood shows, as a rule, marked reduction of red cells, low Hb-index, and moderate but distinct leucocytosis. In dispensaries the earlier stages of the disease are encountered, cachexia and palpable tumors are not always noted, many tentative diagnoses stand with positive cases, and the blood very often fails to show any pronounced alteration.

In a considerable group of cases the progress of the usual anemia is interrupted, however, for unknown reasons, and while the patient emaciates, the blood appears to maintain its standard. Hampeln, Neubert, Dehio, and others, therefore, speak of an **anemic** and a **marantic type** of carcinoma. Neubert's cases, five esophageal and four gastric, were especially adapted to illustrate the differences, but both types may occasionally be seen in other forms of the disease—*e. g.*, cancer of uterus.

Many careful studies have shown that in the earlier recognizable stages of the disease, and in somewhat more advanced phases of visible growths, the blood remains practically normal. Few published series, however, show so large a proportion of cases with red cells above 4,000,000 as does Cabot's, in which thirty-four of one hundred and fourteen cases of carcinoma of stomach gave over 4,000,000 and twenty-five over 5,000,000 red cells. Yet this series may well serve to emphasize the fact that carcinoma commonly exists in otherwise healthy subjects, and for a time fails to reduce the number of red cells. The significance of a normal red-cell count is, of course, limited by the fact that in many situations the growth tends to retard the ingestion and absorption of fluids and thus to concentrate the blood. Yet in some instances the malignant growth, like tuberculosis, appears to exert some obscure *concentrating influence* on the blood, and even at death, although the body is emaciated, the blood may be unusually deep red.

Osterspey found 5,000,000 red cells and 98 per cent. of Hb three months after the symptoms of a gastric cancer were developed, while in a patient who had lost one hundred pounds in weight within a year from gastric cancer he found 4,544,000 red cells and 82 per cent. of Hb. Price-Jones reports a case of long-standing cancer of the breast with extensive general metastases in which the blood at death showed 6,960,000 red cells and 95 per cent. Hb. For one month before death there was a progressive increase in the number of red cells. The patient took very little nourishment. Of 114 cases of gastric cancer Cabot reports over 5,000,000 red cells in 25, and over 6,000,000 in 4. Laache, who noted the same apparent immunity of the blood against the effects of a bleeding uterine carcinoma, concluded that there is an *individual insusceptibility to the effects of malignant tumors.*

It is, nevertheless, true that in the majority of carcinomata the first appearance of cachexia is accompanied by a corresponding oligocythemia. Cancers of the stomach are among the more active in reducing red cells, and some of them, even while remaining of small size, lead to the changes of pernicious anemia. Grawitz counted only 500,000 red cells in one extreme case of gastric cancer. Of complications, hemorrhage and ulceration usually affect the red cells promptly. Rapid growth and numerous metastases also have a similar tendency, but probably less marked.

Morphologically, **the red cells** in carcinoma, while presenting the usual degenerative changes of secondary anemia, do not show any special peculiarities. The usual changes are those of well-marked chlorotic anemia with considerable deficiency of Hb, but with moderate changes in size and shape. Grawitz found granular degeneration of many cells in ten cases of cancer of the stomach or esophagus, but none in two cases of uterine cancer. The writer finds that in those cases in which the appearance of the blood suggests pernicious anemia the deficiency of Hb, even in the majority of megalocytes, is nearly always quite distinct.

Mouisset has also called attention to this fact, finding the Hb-index in these cases to be quite as low as in chlorosis. In a case of Daland's, the hematocrit gave rather fewer red cells than the hematocytometer, but in pernicious anemia the volume of the red cells is usually much above normal (cf. Moraczewsky).

Nucleated red cells are commonly seen when the anemia is severe, and in some cases of gastric cancer they may be extremely abundant. Usually these cells are of normal size, but megaloblasts appear in the severest cases. It has been suggested that the predilection for the bone-marrow as the seat of metastases is responsible for the large number of nucleated red cells seen with many advanced carcinomata (Epstein). They are frequently seen, however, in the early stages of the growth and when anemia is slight. Cabot found them in four-fifths of all the severe cases, Schreiber when the Hb was normal, and Price-Jones when the red cells numbered 6,390,000.

The **hemoglobin** usually begins to suffer before the red cells, and hence the type of anemia seen in the great majority of early cases resembles chlorosis. Fully normal Hb appears to be a good negative indication against carcinoma at any stage. In internal visceral carcinoma this rule is almost invariable. There are, however, reports of 90 to 100 per cent. of Hb in cases with increased numbers of red cells, and the loss is doubtless slight during the early stages of most cases. In carcinoma of the breast there is often no sign of anemia. Usually a malignant epithelial tumor rather promptly reduces the

Hb, so that during the greater part of its progress the Hb is below 75 per cent. From a comparison of results reported by various observers on about three hundred cases it appears that the averages encountered in routine experience are Hb 56 per cent., red cells 3,700,000. In Bierfreund's and Reinbach's series of fifty-seven surgical cases the Hb varied between 18 and 80 per cent., while few registered over 70 per cent., and in comparison with benign tumors the Hb in cancer was distinctly lower. After operation the restoration of the Hb required about a week longer with malignant than with benign tumors, and the restoration was never quite complete. With internal visceral carcinomata the Hb has usually been found even lower, but there are numerous exceptions to this rule, usually depending on the site of the tumor.

A rather uniformly low percentage of Hb in visceral carcinomata was found by Laker, Eichhorst, Haberlin, Laache, Dehio, Leichtenstern, Moraczewsky, and Strauss and Rohnstein (40 per cent.), while Sailer's and Taylor's series of twenty-one cases gave the unusually low average of 25 per cent. Osterspey's and Cabot's cases were somewhat higher, often approaching the normal. *Yet Haberlin concluded that in stenosis of the pylorus cancer is contraindicated if the Hb is over* 60 *per cent.* In cases followed for considerable periods, as by Price-Jones, the Hb in some remained stationary, in others it declined, while in a single case (ovarian carcinoma) it remained normal for six months.

The **Hb-index** is usually low. In some cases it has approached that of chlorosis, and although it is usually somewhat higher carcinoma offers a closer resemblance to chlorosis in this respect than almost any other cause of secondary anemia. As the disease progresses the anemia itself becomes more severe, and when chronic cachexia has been long established the blood shows the characters of secondary pernicious anemia, with variations in the size of the cells and increasing Hb-index. Yet with rare exceptions the relatively great loss of Hb remains characteristic.

Leichtenstern[2] long since called attention to a rapid increase of Hb, to 100 per cent. or more, seen in some cases of gastric cancer shortly before death, and to the tarry appearance of the blood at autopsy. Patrigeon, in a gastric case, found the red cells normal nine days before death. The writer has observed this condition in patients who had failed to take any food and little drink for some days before death.

Special Factors in the Anemia of Carcinoma. The special conditions leading to severe anemia are numerous, and some are obscure. A previous condition of anemia may exist before the development of the cancer, as when a round ulcer of the stomach becomes malignant. The course of the blood changes in many cases strongly indicates that the *previous condition of the patient* and the general constitution are very prominent factors in the anemia of carcinoma. *Hemorrhage and ulceration* are among the frequent complications recognized as rapidly impoverishing the blood, but occasional escape from their natural effects has been recorded. The *site of the tumor* may be such as to interfere with nutrition, as do some gastric carcinomata. *Rapidly forming and numerous metastases* are usually associated with severe and progressive anemia, but there are numerous exceptions to this rule, some emaciated subjects showing a tendency to concentration of the blood. In two cases with extensive bone metastasis the writer found only moderate anemia and but few nucleated red cells. The histological character of the tumor appears to have no relation to the anemia except in so far as the structural type favors hemorrhage or ulceration. The *chronic toxemia* of carcinoma may apparently affect the blood in a variety of ways. Müller offers evidence to show that the blood of cancerous patients contains toxic principles which destroy albumins and diminish alkalescence. Grawitz injected the alcoholic extract of a cancerous tumor into rabbits, and, finding a considerable loss of gravity and dry residue, concluded that the toxins of carcinoma tend to dilute the blood by inducing excessive flow of lymph.

Maragliano has demonstrated an *increased globulicidal activity of the serum* in carcinoma.

The Leucocytes. Leucocytosis in carcinoma was very early recognized as a frequent condition, having been observed postmortem by Andral, in 1823, and in the living blood by Lucke and by Virchow about 1867. The white cells were long regarded as derivatives of the tumor, an error which was slowly relinquished when leucocytes were submitted to more careful classification by Schultze.

That the carcinomatous process has in itself any capacity to draw leucocytes to the blood may still be doubted, although the edges of the tumor are always inflamed and adjacent lymph nodes invariably show inflammatory hyperplasia before metastasis. In tumors of ordinary size and character this inflammatory process is usually too limited to affect the leucocytes, but with very large growths, as in the liver, lung, uterus, etc., the frequency of leucocytosis without complications indicates that a large, actively growing tumor, merely through its local irritation, may induce leucocytosis. Thus Hayem saw a leucocytosis of 21,700 disappear after the removal of a scirrhus of the breast, only to return again with the recurrence of the tumor. Hayem believes that recurrences may be predicted by the appearance of a gradually increasing leucocytosis. On the other hand, advanced cachexia may exist with hypoleucocytosis (Sailer, Taylor, Strauss and Rohnstein). **Rapid growth** is much more effective in raising the number of leucocytes, probably by exciting more active local reaction, and by more sudden disturbance of function in the part affected.

Superficial ulceration, or other inflammatory complications, are the usual causes of well-marked leucocytosis with carcinoma. Hemorrhages usually accompany ulceration, and add distinctly to the excess of leucocytes commonly seen in bleeding and ulcerating tumors.

The **type of the tumor** has a distinct relation to leucocytosis, which, however, cannot always be traced. The cellular carcinomata tend to irritate, ulcerate, soften, and bleed, while fibrous tumors are of slow growth and non-vascular. The writer has observed two very large, gelatinous carcinomata of the peritoneum without leucocytosis. The diffuse cancers of the stomach regularly excite less increase of leucocytes than does the adenomatous type.

The **situation of the tumor** often determines the frequency of leucocytosis from special liability to ulceration or bleeding. Many cancers of the stomach and uterus illustrate this rule. Epithelioma of the esophagus rarely induces leucocytosis unless there is extreme ulceration and extension.

Unusually high leucocytoses are reported in a case of carcinoma of thyroid, by Hayem, and in cases of unidentified tumors of the kidney, by Cabot, and in several cases of gastric cancer (q. v.).

LEUCOCYTOSIS IN CANCER OF THE BREAST.—Alexander found an average of 11,400 leucocytes, and variations between 2360 and 21,700, in fourteen cases of scirrhus. In three cases of alveolar carcinoma with extensive metastasis the leucocytes numbered 10,075, 11,625, and 12,400. Hayem (p. 947) found a distinct reduction of leucocytes following four operations for scirrhus. The chief feature in the numerous counts of Price-Jones is the variability of the leucocytes in cancer of the breast.

It will be seen that the leucocytosis of carcinoma is referable largely to complications, and these complications are such as appear very constantly in rapid or advanced cases. These conclusions accord with the general experience that most cases of well-established carcinoma are accompanied by leucocytosis. The great variety of these complications render it unwise to draw any narrow diagnostic conclusions from the presence or absence of leucocytosis.

The **varieties of leucocytes** in the blood in carcinoma are found in much the same proportions as in other forms of cachectic leucocytosis. With marked leucocytosis the polynuclear cells usually form a high percentage, 77 to 89 per cent. (Reinbach); 74 to 96 per cent. (Cabot). Strauss and Rohnstein obtained an average of 15 per cent. of lymphocytes in thirty-five miscellaneous cases, and these authors believe that a relative diminution of lymphocytes is seen in the vast majority of anemic cases, proceeds uniformly with the disease, and without reference to the total number of leucocytes. Yet in a case of the pernicious anemic type of gastric cancer Sailer and Taylor reported 45,000 leucocytes, of which 46 per cent. were mononuclear, and these authors found the large mononuclear cells to regularly exceed the small lymphocytes. Unusually high leucocytoses have been reported in the anemic type of gastric cancer by several other observers, some instances of which apparently represent antemortem leucocytosis, while in others the excess was noted long before death. Eisenlohr, Mayer, and Lebert reported a ratio of 1 white to 50 red cells; Potain, 1 to 48; Welch, 1 to 20.

Braun has described a case of cancer of prostate with pernicious anemia (1,000,000 red cells, 10,700 leucocytes) in which the majority of white cells were lymphocytes, but a few eosinophile myelocytes were present. Eosinophiles usually persist in low normal proportions, from 1.2, 1.5, to 2 per cent. (Cabot, Sailer, Taylor, Reinbach).

Myelocytes are found in a large proportion of cachectic cases, and sometimes in considerable numbers. They are most abundant in the antemortem leucocytoses, when the excess of various forms of leucocytes, with normoblasts and anemic red cells, may suggest leukemia. Sailer and Taylor found 9.3 per cent. of these cells in a very anemic case. Leucocytes holding fragments of red cells or showing various degenerative changes are described by Hayem and Escherich.

Specific Gravity. The specific gravity of the blood in well-established carcinomatous cachexia is remarkably low. This fact is illustrated in Dieballa's series, in which cases of gastric cancer with 1,500,000 to 2,200,000 cells show the same gravity, 1.030 to 1.032, as cases of pernicious anemia with only 500,000 to 1,000,000 cells. Peiper also found very low gravity in four advanced cases, while the lowest reports are those of Moraczewsky, 1.012 and 1.016. While Hammarschlag obtained nearly normal gravity for the serum in these cases, Grawitz, Strauer, and v. Jaksch found exceptionally low percentages of dry residue and albumin, indicating that in carcinoma the albumins of the serum suffer in a peculiar degree. Biernacki located the marked loss of albumins principally in the red cells, but concluded that the iron is not always markedly diminished.

In the less advanced cases of carcinoma the gravity of the blood does not differ from that of other secondary anemias (Devoto, Schmaltz, Scholkoff, Hammarschlag, Stintzing, Gumprecht).

The **alkalescence of the blood** has been found considerably diminished, as in other forms of secondary anemia. Klemperer and v. Limbeck, Peiper and Rumpf, and Moraczewsky, using different methods, found very low grades of alkalescence in advanced cases. Van Noorden refers this change to the liberation of sulphuric, phosphoric, acetic, and oxybutyric acids, from the destruction of albumins.

An **excess of sugar** was found by Freund in a series of cases, and while Trinkler confirmed these results, Matrai had previously shown that it is neither constant in nor peculiar to carcinoma.

Hemolytic properties of the blood of carcinoma have been demonstrated by various observers. Strauss and Rohnstein found that a few injections of blood serum from such cases kill rabbits with a loss of 2,500,000 red cells and leucocytosis, maximum 23,200, and 83 per cent. of polynuclear cells. Camus and Pagniez and others have found the serum of cancerous patients actively hemolytic for red cells of healthy subjects, while innocuous to the red cells of the patient. Bard and Milian have found the hemorrhagic fluids coming from pleural and peritoneal carcinomata hemolytic to normal human red cells. Lang finds that the hemolytic substance, while responsible for the anemia, leads to increased resistance in the remaining cells.

A **peculiar form of coma**, described by v. Jaksch[1] and others, sometimes terminates the course of gastric cancer, and has been referred by Senator to an obscure toxemia resulting from disturbed metabolism. Herter's demonstration of a great excess of bases over acids, and a great diminution of ammonia in the urine in chronic gastritis, strongly indicates that an acid intoxication exists in these cases, as in diabetic coma. (See Chronic Gastritis.)

SARCOMA.

The early changes in the blood in sarcoma closely resemble those of carcinoma, but some comparison of details will be of interest.

Red Cells. The usual variations in the progress of anemia are to be observed, including the absence of oligocythemia, or even the presence of polycythemia in the early stages (Alexander, Reinbach, Cabot), and very slow or very rapid impoverishment of the blood according to the rate of growth and character of the complications.

In the cases recorded by Hayem, Alexander, Laker, Limbeck, Sadler, Rieder, Reinbach, Bierfreund, Cabot, it is impossible to find any uniform difference in the grade of anemia from that observed with carcinoma, and this conclusion accords with the writer's experience. Reinbach, however, finding only four normal blood specimens in twenty cases of sarcoma and six among sixteen cases of carcinoma, concluded that the anemia of sarcoma is usually greater than in carcinoma.

There are also some pathological grounds on which Reinbach's conclusions may be supported, in the special relation of sarcomatous growths to bloodvessels and marrow, their greater tendency to hemorrhage and degeneration, and their more frequent association with febrile processes, leucocytosis, or even leukemia.

The lowest number of red cells observed appears to be that recorded by Hayem at 663,400, while Rieder found only 6 per cent. of Hb (?) in a dying subject. Limbeck says that normal blood is more frequently found with early sarcomata than with early carcinomata, while Reinbach has never seen in early carcinoma the extreme alterations of the blood which he referred to an early sarcoma. Alexander reports an extensive osteosarcoma of sternum and vertebræ with over 6,000,000 red and 52,000 white cells, and in his and other series of osteosarcoma the average anemia is not extensive.

In Cabot's sixteen cases the average of red cells was 4,400,000, with extremes between 2,600,000 and 6,200,000.

Nucleated red cells are rather less common than in carcinoma. The changes of *progressive pernicious anemia* were present in the blood in cases of osteosarcoma reported by Grawitz, Ehrlich, Mosler and Gast, Fede, and Hausler.

Leucocytes. The relatively greater frequency and extent of leucocytosis in sarcoma appears to be the most striking difference in the blood between the two groups of malignant tumors. The great majority of sarcomata when first observed have shown a distinct leucocytosis, which, moreover, tends to persist and often to increase, with the advance of the tumor, until at death the upper limits of inflammatory leucocytosis are frequently reached. Martin and Matthewson have described leucocytosis of this extreme type and pointed out some of the difficulties in diagnosis which may result therefrom.

Indeed, there are several recorded instances in which the lymphocytosis of sarcoma *terminated in lymphatic leukemia.* The writer observed such a case in 1899, and others have been reported by Palma, Sadler, and Strauss.

Palma's and Sadler's cases were round-celled sarcomata. In the writer's case various parts of the tumor and its metastases showed gradations between round and large spindle-celled sarcoma. The recorded transformations of pseudoleukemia into leukemia are doubtless of similar significance (Fleischer, Penzoldt, v. Jaksch,[3] Westphal, Mosler, Senator).

The **types of leucocytes** show greater variety in sarcoma than in carcinoma. The majority of leucocytoses being of inflammatory origin, an excess of polynuclear cells is the rule, and even with a normal number the proportion of these cells may be excessive. LYMPHOCYTOSIS, though not relatively frequent with sarcomata, has at times reached the grade of lymphatic leukemia, as in the cases mentioned. In lymphosarcoma, as in pseudoleukemia, the lymphocytes are not usually excessive, and in many cases are very scanty. EOSINOPHILIA has been regarded by Neusser as one of the diagnostic signs of sarcoma of bone-marrow. A marked and persistent excess of these cells, 48 per cent., was found in one of Reinbach's cases of lymphosarcoma complicated by phthisis, and bone metastases were found at autopsy. Three other cases, without autopsy, showed from 8 to 12 per cent. of eosinophiles, but these cells were normal in most of his cases, and absent in five.

There seems to be some difference in the extent of leucocytosis observed in various types of sarcoma. In sixteen cases of osteosarcoma collected by Cabot from several authors the average was 17,000, in twelve lymphosarcomata 20,000, and in seven melanotic sarcomata 25,100. Without further details than the reports of these cases furnish, the significance of these data is limited. The greater leucocytosis of melanotic sarcoma accords with its recognized malignancy, and some marked leucocytoses observed by Alexander and Limbeck (52,000 and 32,000) in osteosarcomata may perhaps go with myeloid tumors, which are more malignant than the periosteal.

Myelocytes were noted in Reinbach's case, cited above, which also showed eosinophilia. Cabot found 7 per cent. of large and small myelocytes in a case of general sarcomatosis, and an occasional myelocyte in three other cases.

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PART VI.

ANIMAL PARASITES.

CHAPTER XXIII.

MALARIA.

I. TECHNIQUE.

(*a*) **The Examination of Fresh Blood.** By far the readiest method of determining the presence of malarial infection is by the examination of the fresh blood. For this purpose thoroughly cleaned and polished slides and cover-glasses are the chief requisites, and a thin layer of blood may be secured by touching the exuded drop to the cover-glass, which is immediately laid upon the slide and examined with a one-twelfth immersion lens. The quantity of blood should be small, and in the resulting layer the red cells should be separated from each other. Besides the facility in preparation, this method permits the study of the vibratory motion of pigment, of ameboid motion of the parasite, of some limited phases of the natural development of the parasite, such as exflagellation, and of some artificial changes, such as the escape of the parasite from the cell, and occasionally of the formation of vacuoles.

By mixing the blood with ascitic fluid deeply tinged with methylene blue, Celli and Guarnieri secured excellent stained specimens of the parasite in the fresh condition. The ascitic fluid was prepared by dissolving powdered methylene blue in the fluid and filtering. The blood was mixed with this fluid and spread under a cover-glass in the usual way. Their drawings of parasites seen under these conditions appear to have been the first definite representation of the nuclear body of the malarial organism. No other notable modification of the method of examining malarial blood in the fresh condition appears to have been perfected.

Concerning the value and reliability of these methods opinions are somewhat at variance. Most of the early study of malarial blood was made exclusively by this method, and it still remains, of course, the only method, permitting the minute study of many important changes in the form of the parasite, of the motility of the pigment, and of some degenerative processes. On the other hand, exclusive reliance on this method appears to have been responsible for much confusion regarding the varieties of the parasite and its minute structure, as well as for many erroneous interpretations of exceptional or artificial changes in the organism.

As a ready means of diagnosis, in the hands of an expert, the examination of fresh blood is all that is required in the average case, and when the parasites are moderately numerous and when crescents are present, even an inexperienced observer can hardly err. Under any other conditions, except for special purposes, reliance upon this method appears to the writer inadvisable. When the parasites are scarce, especially when they are of the small, unpigmented form, a prolonged search through fresh blood has frequently proven negative, in the writer's experience, although a few minutes sufficed for the discovery of one or more minute parasites in the stained specimen. Practically, this fact demands that a negative result with a fresh specimen be invariably controlled by the examination, with mechanical stage, of a stained specimen. Moreover, anyone who is familiar with the host of appearances in fresh blood simulating the malarial parasite must regard with some suspicion the report, from anyone but a recognized expert, of the discovery of one or two "hyaline bodies." From these considerations the writer would limit the use of fresh specimens to the study of a few special features of malarial parasites, and would urgently recommend that as a diagnostic procedure reliance be placed only upon the examination of dry specimens stained, preferably, by Nocht's method.

The study of flagellate bodies may be conducted in fresh specimens prepared in the ordinary way, but placed, if possible, on a warm stage. Mannaberg alone claims to have found the flagellate bodies in considerable numbers in the fresh human blood immediately after shedding. Usually they appear only after the lapse of ten to twenty minutes, when they may form in variable numbers from the larger tertian or crescentic estivo-autumnal organisms, less frequently from quartan parasites.

The addition of a little water or salt solution may facilitate the escape of the parasite from the red cell and the formation of flagella.

The successful action of moisture has been obtained by several expedients. Marshall added about an equal quantity of water to a small drop of blood containing many crescents and saw the almost immediate change of crescents to spheroidal bodies, followed by exflagellation. Manson recommends that the blood under the cover-glass be kept moist by exposure to steam exhaled from a hot moist sponge. After a few minutes the cover may be carefully removed, the specimen dried, and the flagellate bodies stained.

The writer finds that the moist chamber may be secured in a Petri dish with tightly fitting vaselined cover. Wet blotting paper placed in the dish furnishes the necessary moisture. Specimens spread on slides or covers may be kept moist for ten to twenty minutes in such dishes, and flagellation proceeds with moderate rapidity.

A simple method is as follows: Cut an opening $\frac{1}{2}$ x 1 inch in a piece of thick blotting paper and moisten the paper in hot water. Spread two glass slides rather thickly with fresh blood, lay the blotting paper on one slide, cover the cut opening by the other, specimen side down, and slip a rubber band about both. After fifteen to twenty minutes the slides and paper may be separated and the two specimens dried.

Far more important than any of these details is the stage of the infection in which the patient is found. No amount of manipulation will succeed in inducing the changes of exflagellation in parasites which are not capable of them. In the early paroxysms of initial infections attempts to obtain flagellate bodies almost always fail, while in the late stages of relapsing cases with rich infection, in which the sexes of the parasite have become differentiated, flagellate bodies form readily and in large numbers.

(*b*) **Preparation of Dry Specimens.** Smears of the blood may be prepared by any of the usual methods. After drying thoroughly in the air the malarial parasite stains best after fixation in 95 to 97 per cent. alcohol for fifteen to thirty minutes. Five minutes' fixation will suffice, but if left in alcohol over night the staining qualities of the organism will sometimes be found slightly altered. When slides have been kept in alcohol, or in the air, after fixation by heat or alcohol, for periods varying from one month to three years, there is a progressive loss of the capacity to unite with eosin, while methylene blue gives a deep, diffuse stain. This tendency may be gradually corrected by passing the slides rapidly through a $\frac{1}{10}$ per cent. solution of nitric acid.

Fixation by heat is less satisfactory for the present purpose, and the addition of ether to the alcohol secures no advantage.

Staining Methods. **Eosin and Methylene Blue.** For ordinary purposes staining by eosin and methylene blue may be generally recommended, and many of the detailed studies of the parasite have been based upon specimens stained in this way. The solutions required are: (1) a saturated alcoholic solution of Ehrlich's blood eosin, diluted with an equal quantity of 95 per cent. alcohol or a 1 per cent. solution in water of Grübler's yellow, water-soluble eosin, and (2) a saturated watery solution of Ehrlich's rectified methylene blue at least one week old.

A light staining by eosin, such as is given by the diluted solution of eosin, is essential for the clear demonstration of the parasite by methylene blue, and in specimens containing only the small signet-ring forms, heavy staining by eosin may almost entirely prevent the subsequent action of methylene blue, and these minute parasites may be overlooked.

By intense staining with old solutions of alcoholic eosin which have absorbed water, fragments of the red cell probably containing traces of Hb may often be demonstrated in the meshes of the parasite, of the presence of which no indication is given by the usual method.

Methylene blue fails to stain the young ring forms, especially of the tertian type, as clearly as is desirable, and more powerful basic staining fluids may well be employed for this purpose. Nocht's method may be recommended over any other, as it facilitates the identification of the small rings by means of a large, densely stained nucleus, but when this method cannot be employed one may resort to the method modified by Futcher and Lazear from the suggestions of Benario and of Marchoux, as follows:

"Fix the specimens five minutes in 95 per cent. alcohol, to 100 c.c. of which has been added 1 c.c. of formalin. Stain one to three minutes in the following mixture: saturated alcoholic solution thionin, 20 c.c.; 20 per cent. carbolic acid, 100 c.c. The fixing solution must be used fresh, and the staining fluid must be at least one week old. The rings are then densely stained and the specimens do not fade."

The sharpest demonstrations of minute, ring-shaped parasites secured by the writer were obtained by staining one hour in diluted Gage's hematoxylon before treatment by eosin and methylene blue. Hematoxylon stains the nucleus of the ring and makes the body of the parasite blacker after methylene blue. Such specimens are specially suitable for photography.

The Nocht-Romanowsky Method. The method originally devised by Romanowsky, and which has given such uncertain results in the hands of many investigators, has now been modified most successfully by Nocht, whose procedure gives positive results without much dependence upon the quality of dyes or the time of staining. (The interesting history of this stain may be found in the writer's article on "Malarial Parasitology," *Journal of Experimental Medicine*, 1901.)

Nocht's modification consists in the addition of a few drops of neutralized Unna's polychrome methylene blue (Grübler) to the 1 per cent. solution of ordinary methylene blue. The usual specimen of polychrome methylene blue is distinctly alkaline, and to be rendered effectual for the present purpose Nocht found that it requires neutralization, preferably by acetic acid. This may be done by adding drop by drop of dilute 2 to 3 per cent. acetic acid until the commercial fluid polychrome blue no longer turns red litmus blue above the zone coming into immediate contact with the dye. The writer has never failed to secure a good result by the following procedure:

1. To one ounce of polychrome methylene blue (Grübler) add five drops of 3 per cent. solution of acetic acid (U. S. P., 33 per cent.).

2. Make a saturated 1 per cent. watery solution of methylene blue, preferably Ehrlich's (Grübler) or Koch's, dissolving the dye by gentle heat. This solution improves with age, and should be at least one week old.

3. Make a 1 per cent. watery solution of Grübler's *watery* eosin.

The mixture is prepared as follows:

To 10 c.c. of water add four drops of the eosin solution, six drops of neutralized polychrome blue, and two drops of 1 per cent. methylene blue, mixing well. The specimens, fixed in alcohol, or by heat, are immersed, specimen side down, for one to two hours, and will not overstain in twenty-four hours. The density of the blue stain may be varied to suit individual preferences. The above proportions need not be rigidly followed, but the polychrome solution should be accurately neutralized, and *the staining mixture should be deep blue.*

Nocht later reports that the two solutions of methylene blue may be replaced by a 1 per cent. solution of Ehrlich's methylene blue, alkalinized by ½ per cent. of NaOH and kept a few days in a thermostat at 50° C. This is the ordinary laboratory method of improvising polychrome methylene blue. To 2 c.c. of water add two to three drops of 1 to 2 per cent. watery eosin, and drop by drop of the alkalinized methylene blue until the original red color of the eosin has almost disappeared. In this fluid specimens stain in five to ten minutes. This method is less reliable than the former.

The rationale of the Nocht-Romanowsky method is not yet fully understood, but it appears most probable that a staining agent which unites selectively with chromatin exists ready formed in polychrome methylene blue, and may be developed in specimens of methylene blue in various ways, among which is slow digestion with an alkali and heat. Nocht refers to this principle as "red from methylene blue." It may be extracted from polychrome methylene blue, etc., by chloroform (Nocht). Michaelis describes its relations to methylene blue, and calls it "methylene azur." It can be obtained from Grübler under the name of "azur-blau," and may be employed in the following watery mixture recommended by Laveran: 0.1 per cent. eosin, 2 parts; aq. dest., 8 parts; 1 per cent. azur II., 1 part. Wash in water and then in 5 per cent. sol. tannin.

Goldhorn has succeeded in digesting methylene blue with saturated solution of lithium carbonate so as to develop in it a large proportion of the red chromatin-staining principle. This fluid, neutralized by acetic acid, not only stains the chromatin rapidly (fifteen to sixty seconds), but demonstrates, better than has yet been done, early and extreme granular degeneration of the infected and other red cells. Goldhorn's blood stain in methyl alcohol fixes and stains at the same time, and may be procured from New York dealers. (See *Transactions New York Pathological Society*, February, 1901.

One who wishes to make the stain himself from methylene blue should follow the directions given by Wright.

Nocht's method furnishes so much information regarding the minute structure of the parasite, and renders its identification so complete and positive, that it must be recommended above all other methods of staining the malarial parasite. Moreover, it has a large field of application in the study of nuclear structures in various other micro-organisms.

II. MORPHOLOGY.

The Tertian Parasite. 1. **The youngest form** of the tertian parasite seen in the red cell is identical in appearance with *the spore* of the parent rosette. It is a compact, spheroidal or slightly oval, or irregular body, about 2μ in diameter. It shows an outer rim of basophilic protoplasm inclosing a single large nuclear body which is achromatic to methylene blue, but stains readily in hematoxylon or by Nocht's method, and which is usually enclosed or accompanied by a clear achromatic substance or space, termed by Gautier "the milky zone." (Plate XIV., Fig. 1.)

In the fresh condition these bodies are noticeably refractive, especially the nucleus, change their position but rarely their shape, and are never pigmented. From the earliest period of infection the red cell is often swollen.

2. **The Young Tertian Ring.** Within a few hours after the chill the parasite is usually found to have assumed a somewhat characteristic ring shape, which it commonly maintains in some definite form up to the pre-segmenting stage. In the late stages of the infection many young tertian parasites do not assume the ring form, but remain compact. (Plate XVIII., Fig. 4.) It seems probable that these forms develop into male flagellating bodies.

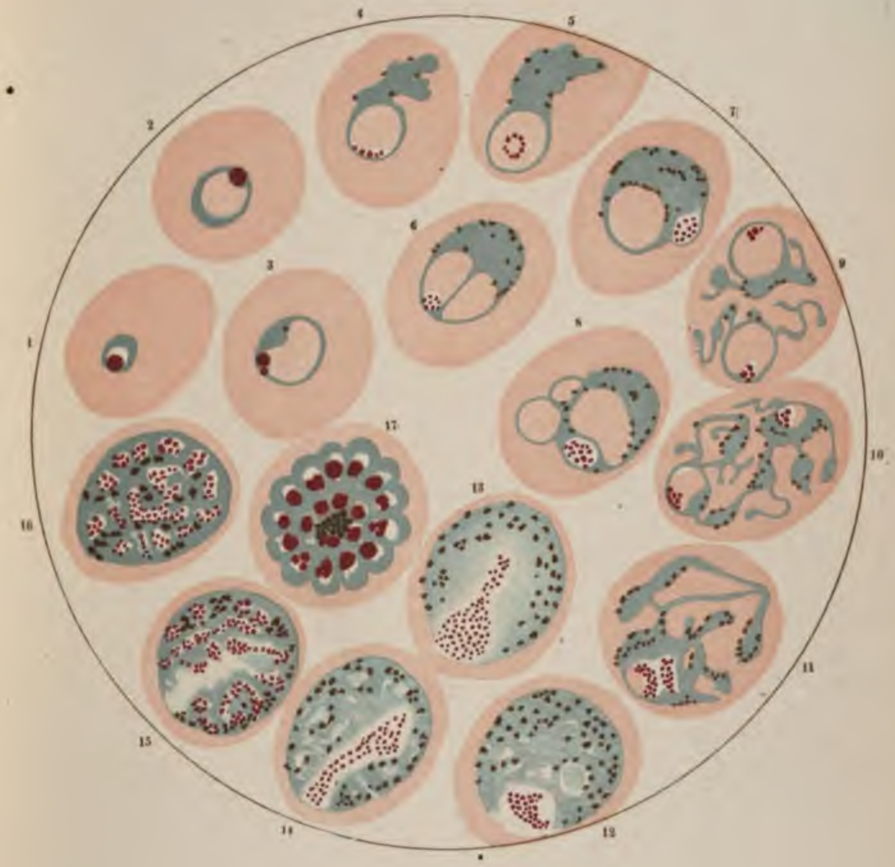
These (ring-shaped) bodies measure from 3μ to 4μ in diameter, and the regular ring form is retained without marked increase in bulk at any point for six to eight hours. Sometimes the ring is elongated, one arm reaching across the cell, while a thin bow persists. Occasionally the ring appears to unfold, and the parasite stretches clear across the swollen cell, with the nucleus at one end. The tertian ring is rarely as geometrical or delicate as the estivo-autumnal signet-ring. The development of pigment is inconstant, some large rings failing to show pigment, but usually one or more fine grains are to be seen in the medium-sized and smaller forms. The ring always encloses a considerable mass of hemoglobin. The nuclear body of the tertian ring is its most characteristic feature, appearing as a rather large, achromatic, highly refractive body after methylene blue, but staining intensely with hematoxylon and by Nocht's method.

Significance of the Ring Form.—In regard to the formation and significance of the ring, opinions are at variance. Most of the Italian writers hold that the ring form is not really a ring, being bridged across by a transparent and vesicular nucleus. There are many considerations favoring this view, especially the usual appearance of the parasite in the fresh condition, and the fact that the chromatin usually lies within the ring, eventually filling it. On the other hand, Mannaberg and Ziemann claim that this body is a true ring, formed by the thinning of the central portion of the body of the parasite, and state they have seen the ring develop in this manner in the fresh blood. From the examination of the rings themselves the writer has been unable to convince himself as to which view is correct, but there are some early forms of the parasite which strongly indicate that the ring does not represent a vesicular nucleus. In one such form the ring is *unfolded* and the nuclear body of the parasite lies naked in the hemoglobin. Moreover, elongated forms of the young parasite are often seen in which the ring is absent and the nuclear body lies bare at one end. These forms vividly recall the appearance of the *ameba dysenteriæ* in which the nucleus remains at the hinder end during active movements of progression. Further, it is difficult to associate the relatively huge size of the ring with any nuclear structure, which would require the young malarial parasite to have a nucleus which is larger than that of the adult *ameba dysenteriæ*. Again, secondary rings sometimes form from the union of pseudopodia, and these are identical in appearance with the primary ring, but lack the chromatin granules.

In specimens stained by Nocht's method the chromatin is usually found within the ring, sometimes lying in an isolated position in the centre, but *very often* the chromatin is found *outside* of the ring, connected by a very fine thread of protoplasm. If the ring represents a vesicular nucleus, we have here the anomaly of a complete separation of chromatin from the vesicular portion of the nucleus, which is opposed to some rigid histological principles. Even more frequently the chromatin is found to be enclosed by bluish staining protoplasm which shuts it off entirely from the ring. (Plate XIV., Fig. 7.)

From various biological studies it appears that the nuclei of the protozoa are usually widely different from the nuclei of metazoa. Many protozoa do

PLATE XIV.



Developmental Cycle of Benign Tertian Parasite.

Fig. 1. Very early form of parasite, showing chromatin granule, "milky zone," and spheroidal body.

Figs. 2, 3. Typical young ring-shaped parasites.

Figs. 4, 5. Subdivision of chromatin, development of body, and appearance of pigment in later ring-forms.

Fig. 6. Double rings in single parasite.

Figs. 7, 8. Turban-shaped parasites. Secondary rings, eccentric position of chromatin.

Fig. 9. Double infection of cell.

Figs. 10, 11. Complex ameboid figures in doubly infected cells.

Fig. 12. Full-grown form, with large eccentric nucleus.

Figs. 13, 14. Protrusion of chromatin granules and milky substance in body of full-grown parasite.

Figs. 15, 16. Division of chromatin granules into groups in reticulated presegmenting bodies.

Fig. 17. Tertian rosette.

not have a vesicular nucleus, with cell membrane, linin, nucleolus, etc., but possesses the so-called "*distributed nucleus*" composed of a number of granules lying free in the body of the parasite. The study of the malarial parasite by Nocht's method indicates that the nucleus of this protozoan is of the *distributed type*, which does not exhibit a vesicular appearance nor possess a nuclear membrane. (See Calkins on "Protozoan Nuclei," *Annals New York Academy Sciences*, 1898, vol. ix., part iii.) On these grounds the writer is inclined to agree with Mannaberg and others who hold that the form in question is a true ring, a form usually but not necessarily assumed by the parasite, and does not represent a vesicular nucleus.

Marchiafava and Bignami still hold that the ring is bridged over by an invisible membrane. They do not insist, as do many, that this membrane is a nuclear structure, but consider the central area to be a digestive vacuole.

Comparison of the Tertian and Estivo-autumnal Rings. From the study of the ring-shaped tertian parasite and the estivo-autumnal signet-ring forms, in typical cases of these infections, the writer believes that these parasites, with very rare exceptions, can be fully distinguished from each other in this early stage, and on the following peculiarities :

1. The nuclear body and chromatin mass of the young tertian parasite is achromatic to methylene blue, which densely stains the nucleus of the estivo-autumnal organism. The writer has been unable to find in the literature any specific reference to this diagnostic peculiarity, which may be readily verified by comparing specimens of the two parasites stained by methylene blue and by Nocht's method.

The dense staining by methylene blue of a nuclear body in the young estivo-autumnal parasite has often been noted, and in 1894 Okintschitz mentioned the fact that the nucleus of the young tertian parasite fails to stain by methylene blue.

2. The shape and contour of the tertian ring is usually coarse and irregular, but the estivo-autumnal ring is geometrically circular, more delicate, with an extremely fine bow, and usually with a typical signet-like swelling. (Plate XIV., Figs. 2, 3, and Plate XVI., Figs. 6, 7.)

3. One or two grains of pigment are almost invariably found in the early tertian ring, but are, with nearly equal constancy, absent from the estivo-autumnal signet-ring.

4. The writer's specimens confirm the statement of Gautier that the tertian ring is usually pigmented before the chromatin becomes subdivided, while the chromatin of the estivo-autumnal ring is always subdivided before the appearance of pigment. In some cases, however, the chromatin of the tertian ring divides before pigmentation.

5. The infected cell is usually swollen from the moment of infection by the tertian spore, and commonly shrunken when harboring the estivo-autumnal ring.

All of these characters are usually apparent in ordinary specimens, but naturally are most distinct in flatly spread and rapidly dried cells. The writer has met with no exceptions to the above rules in cases infected by the tertian parasite in New York and cases of estivo-autumnal infection from Cuba. In many of the irregular relapses, in cases showing tertian organisms, encountered among volunteer soldiers long returned from Cuba, single, ring-shaped parasites not admitting of positive identification have sometimes been seen.

3. **Large Tertian Rings.** After a period of six to eight hours the tertian ring is usually found to have developed an outgrowth which is actively ameboid in the fresh condition, and appears in stained specimens as a tongue-like protrusion or turban-shaped mass attached to one segment of the ring. (See Plate XIV.) The nuclear body meanwhile increases slightly in size, projecting into the ring, and the chromatin divides into several large granules.

At this period occurs the greatest ameboid activity of the parasite, and in some severe tertian infections the organism may be found fixed in the height of its ameboid excursions, when it presents in stained specimens the peculiar appearance depicted in Plate XIV., Figs. 9 and 10. There the ring persists, but the body of the parasite is strung out into a number of slender threads with nodal thickenings. At times, especially in cases taking quinine, the number and delicacy of the threads greatly exceed those seen in the sketch, which represents an average instance.

A close inspection of cells harboring such parasites may disclose the presence in one cell of two distinct nuclear bodies, indicating the co-existence of two parasites. Frequently twin parasites entirely separate from the other are found in the same cell, each of which shows a tendency to develop the long threads. (See Plate XIV., Fig. 9.) When, however, the threads are numerous and very thin it is usually impossible to find any break in their continuity, while in many instances the two parasites are distinctly united. The significance of these forms will be considered later. (See Conjugation.)

4. **Tertian Spheroidal Bodies.** *During the second quarter* of the cycle the body and the nucleus of the parasite develop rapidly in size, ameboid motion and ameboid figures gradually diminish, and pigment is abundantly deposited in the form of fine dark-brown or yellowish grains showing in the fresh state active vibratory motion. The infected cell continues to increase slightly in size, and its hemoglobin is progressively diminished.

Depending upon the character of ameboid activity, the variety of figures seen during this period is very great. Eventually, toward the end of twenty-four hours or possibly somewhat later, the parasite occupies three-fourths of the swollen cell, in the form of a spheroidal or elliptical, homogeneous body, the outer portion of which contains most of the pigment and is rather more deeply stainable than the zone immediately surrounding the nucleus. (Plate XIV., Fig. 12.) The nucleus gradually increases in size, growing into the ring. It no longer has the appearance of a small, highly refractive, achromatic spot (after methylene blue), but takes a light bluish tinge with 1 per cent. methylene blue, and stains less deeply than before with hematoxylon. At the end of this period the nucleus completely fills the ring, stains rather distinctly with methylene blue, and sometimes exhibits a delicate bluish network.

After Nocht's method the changes in the nucleus are found to consist in the gradual subdivision of the chromatin granules, which finally become rather numerous, of minute size, and more difficult to stain.

Usually these chromatin granules lie on the inner circumference of the bow of the ring, projecting within the ring, and partly surrounded by a "milky," unstained space. This clear zone is often absent in young parasites in cells thinly spread and rapidly dried, but in older parasites it is always present. Various other positions may be assumed by the chromatin mass, as follows:

A subdivision of the granules into two distinct groups, separated by a strand of bluish-stained protoplasm; an excentric position entirely apart from the ring; a position midway between two rings found in the same parasite; a position in the centre of the ring entirely apart from any bluish protoplasm; a circular arrangement about the periphery of the ring. Sometimes the smaller granules are grouped about a central, larger granule, as has been noted in other protozoa whose nuclei are of the intermediate type (*Microglena*, *Euglena*). (Plates XIV., XVI.)

5. **Full-grown Tertian Parasites.** *The third quarter* is occupied by the continued growth of the parasite in the form of a large, homogeneous, richly pigmented body, which finally occupies at least four-fifths of the swollen cell, and by certain nuclear changes which it is difficult to follow in specimens stained by methylene blue or hematoxylon, but are fully demonstrated by Nocht's method. The exact limits within which the parasite may be termed "full grown" can be sharply fixed only with great difficulty, but there appears to be a period of at least twelve hours during which there is little change in the structure of the organism, and during which the body stains homogeneously and the nucleus occupies the entire ring. The writer would place this period between the twenty-fourth and fortieth hour of the cycle. A portion of the period is occupied by nuclear changes belonging to the reproductive phase of the parasite's development.

After the appearance of a faint intranuclear network most authorities agree that the nucleus largely disappears in specimens stained by methylene blue or hematoxylon, and is next seen in the form of highly refractive achromatic spots in the meshes of the reticulated pre-segmenting body (methylene blue), and these again stain deeply with hematoxylon.

Nocht's method, however, fully demonstrates the nuclear changes which occur in the full-grown parasite. A considerable area, usually the entire original ring, is now occupied by a "milky" or slightly bluish staining substance, in which lie a considerable number of very fine chromatin granules. These granules are usually difficult to stain, and being of very minute size they are difficult to see. This fact has led Ziemann and Gautier to admit the possibility that the chromatin may actually disappear at one stage of the development, especially since they have found some large parasites in which no chromatin was demonstrable. In the writer's specimens there were a very few large tertian parasites in which no chromatin granules appeared, but these were not more numerous than younger forms which were devoid of chromatin and which must, therefore, be regarded as sterile. Similarly, the larger forms devoid of chromatin the writer would class with the sterile forms rather than accept the view that the chromatin may entirely disappear at one stage of the fertile parasite, a view which is at variance with biological principles. After the subdivision of chromatin has reached a limit, the next change, observed in a considerable number of parasites, appears to consist in the extrusion of a portion of the milky substance and its chromatin granules into the body of the parasite. (Plate XIV., Fig. 13.) At the same time the granules of chromatin increase in size and diminish in number. Other forms may be seen in which the "milky substance" and chromatin granules occupy an elongated space within the body of the parasite, and in such cases the beginning concentration of pigment and deeper stain of the parasite indicate the presence of the pre-segmental stage. (Plate XIV., Fig. 14.)

Considerations pointing to the subdivision of the group of full-grown tertian parasites are mentioned in the section on Conjugation.

6. **Pre-segmenting bodies** usually begin to appear in the blood eight to ten hours before the chill.

In specimens stained by methylene blue the first demonstrable indications of the division of the parasite are seen in a *deeper staining capacity* and *tendency toward reticulation* which appear throughout the whole or in a part of the body of the parasite. Occasionally these changes may be noted in one-half the parasite, while the other half retains the homogeneous apperance of the "full-grown" organism. Usually the process is found to have affected the entire organism, giving the very characteristic forms sketched in Plate XIV., Figs. 15 and 16. In the pre-segmenting bodies the pigment is gathered in a reduced number of coarse grains or spindles which lie in the body of the parasite, in a position determined by that of the new multiple nuclei.

These bodies were first described by Golgi in fresh blood and properly interpreted as belonging to the process of segmentation. Later they were described by Marchiafava and Celli as vacuolated parasites, the highly refractive nuclear bodies appearing in the fresh condition very much like vacuoles. Still later, Celli and Guarnieri sketched them from specimens stained in the fresh condition, regarding some as showing partial segmentation, others as vacuolated parasites, although they accurately described the appearance of the nuclear particles invariably found within these "vacuoles," while others they supposed to be groups of confluent parasites, *i. e.*, true plasmodia. Mannaberg's descriptions (1899), referring only to fresh blood, do not include these bodies, nor have they found a distinct place in his plates, although some of the figures in Plate IV. indicate that they have not escaped his observation. In Thayer and Hewetson's careful study of the parasite in fresh blood, the pre-segmenting bodies are not described as such. Laveran (1898, page 62) refers to the similarity in appearance between a nuclear body and "vacuole," but he neither describes nor depicts the pre-segmenting reticulated parasite. Ziemann describes the pre-segmenting bodies as they appear after his or Romanowsky's staining methods, but the plates would not enable one unfamiliar with the subject to identify these forms in specimens stained by eosin and methylene blue.

The reticulated, pre-segmenting, tertian parasites may be seen in every case examined within six to eight hours preceding the chill, and often in belated parasites shortly after the chill. Many transitional stages between the homogeneous adult parasite and the perfect rosette may be seen in rich infections. They are well demonstrated by eosin and methylene blue, especially as regards the increasing density of stain and reticulation. After hematoxylon the multiple nuclei stain deeply.

By Nocht's method a series of interesting nuclear changes may be followed in the pre-segmenting forms. After the mass of enlarging chromatin granules and milky substance has flown out into the elongated form described above, the chromatin granules leave the central clear space and make their way in groups out into the body of the parasite. Various stages of this process may be followed in specimens taken at suitable periods, and some observed phases are seen in Plate XIV., Figs. 13 to 15. Considerable differences in the number of such groups may be noted in different cases. Usually a large number of ill-defined groups are seen before the central mass of granules is exhausted. (Plate XVI., Fig. 14.) In some specimens the compact nuclei of the young spores appear to form in one segment of the parasite before the main mass of granules has become exhausted. Each of the groups appears always to be surrounded by a milky zone, and the mass of granules is often of a peculiar triangular form. During these changes the pigment granules increase in size, diminish in number, and are distributed in the meshes of the now distinctly reticulated body of the parasite.

In the late stages of infection rather small but full-grown forms may be found with four to six groups of chromatin granules. It seems probable that these bodies are destined to flagellate, and are developed from the compact, densely staining forms of younger parasites.

7. **Tertian rosettes** are usually seen in the circulation three or four hours before the chill, most abundantly just before the chill, and a few are often to be found for one hour or longer after the chill.

These limits may occasionally be much wider, as Marchiafava and Celli have seen rosettes two to six hours before the chill and six to seven hours thereafter, and, indeed, when the different broods of parasites are not very distinct, there is no reason why occasional rosettes should not be found at any period of the main cycle.

Of the three types of sporulation described by Golgi, the second type, according to which the entire parasite is divided into spores, leaving nothing but pigment, is undoubtedly the usual process. As regards Golgi's first type, after which only the peripheral portion of the parasite divides, leaving a distinct central, globular, pigmented body, most stained specimens fail to show convincing evidence that the physiological segmenting process may be subject to such an important modification, nor does it appear in recent literature that the existence of this variety of segmentation has been fully verified. In a few cases taking quinine the writer has seen rare segmenting bodies which resembled those described as above by Golgi, but never in fresh cases. Golgi's third type of "partial segmentation," together with the lateral circumscribed sporulation of Celli and Guarnieri, may frequently be seen in rich tertian infections in fresh blood, but, according to the evidence of stained specimens, must be classed with the pre-segmenting forms. The various morphological differences seen in the segmenting forms of the separate species serve to distinguish those species, but do not constitute different types of this process.

The tertian rosette is usually distinguished by its large size and considerable number of spores—fifteen to twenty. From the writer's specimens it does not appear, however, that the identification of the tertian rosette can always be based upon the number of spores, as these are sometimes found to number under fifteen, though rarely over twenty-one. Marchiafava and Bignami, however, have described tertian rosettes with forty to fifty spores. With the smaller number of spores the rosette is always distinctly larger than either the quartan or the estivo-autumnal body.

Although rosettes sometimes appear to be extracellular when seen in fresh blood, in stained specimens the writer has never seen a clearly extracellular segmenting tertian body. Almost invariably there is an unbroken ring of hemoglobin about the parasite, and very often traces of hemoglobin may be found scattered through a mass of spores, where they may be demonstrated by dense staining with eosin.

The nuclear changes demonstrated by Nocht's method in the tertian rosette consist principally in the gradual fusion of the new-formed groups of chromatin granules into one compact globule, which is partly surrounded by a "milky zone," which is probably an artifact.

While the rosette is still compact the vesicular shape of the spore is distinct. The outer segment of the ring is usually thickened, the nucleus tends to lie near the inner pole, and between the nucleus and the outer segment is a small, milky zone.

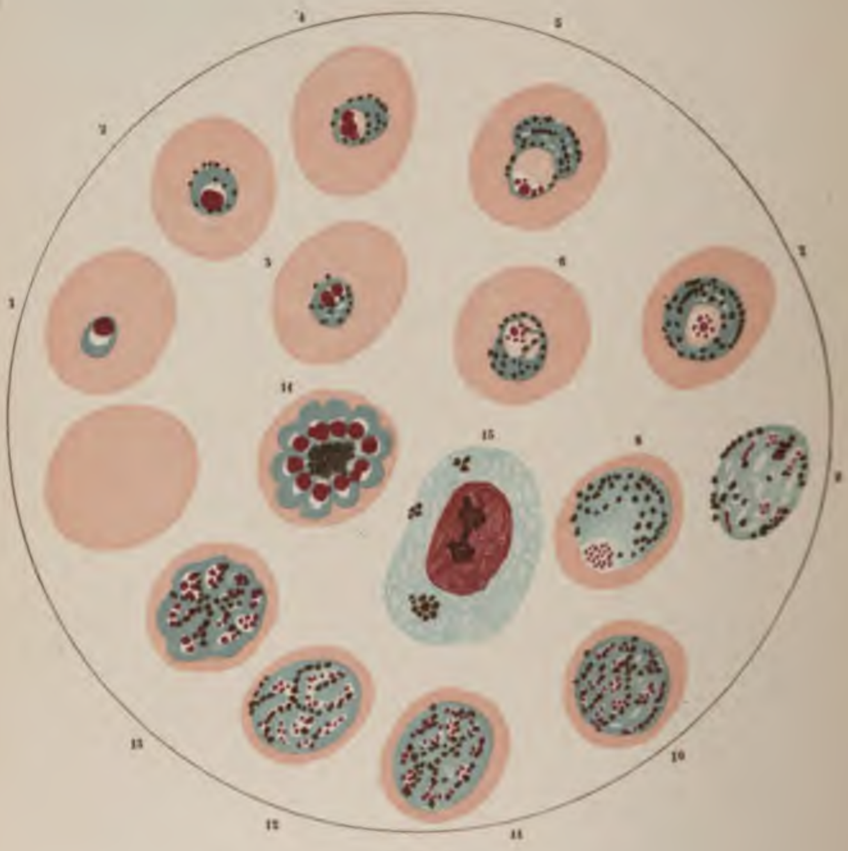
The pigment is usually collected into a central block or mass of granules, but may be found variously scattered among the spores, or along the periphery of the rosette. (Plate XIV., Fig. 17.)

The Quartan Parasite. The earliest form of the quartan parasite as seen in the stained red cell is practically indistinguishable from that of the tertian organism, but its true character may usually be suspected from the slightly shrunken appearance of the infected cell. In fresh specimens the higher refractive quality of this body is often, however, sufficiently characteristic for its identification. After a very slight increase in size the quartan parasite becomes rather easy to distinguish in both fresh and stained specimens, for it early takes the form of a ring, of the general character of the tertian ring, but smaller, more compact, and more richly and coarsely pigmented. As with the tertian organism the nuclear body is found projecting into the ring. In fresh specimens at this period the higher refractive quality and slower ameboid motion are additional diagnostic characters from the tertian parasite. (Plate XV.)

The growth of the quartan ring is very similar in all important respects to that of the tertian, while its distinguishing features, especially the abundance of coarse pigment grains, are uniformly retained.

During the pre-segmenting stage the characters of the quartan parasite are markedly different from those of the tertian. On account of the slower progress of sporulation, and from the greater tendency of the quartan parasite to complete its cycle in the general circulation, quartan pre-segmenting bodies are relatively much more numerous in the stained specimen than are the similar forms of the tertian organism. In some specimens taken several hours before the chill the majority of organisms found may present the markedly reticulated structure indicative of approaching division. The multiple nuclei being less numerous and the pigment more abundant, the meshes of the reticulum are much coarser and the pigment is often found in irregular, partly radiating rows. These coarsely reticulated, relatively small, and richly pigmented bodies, lying in markedly shrunken cells, are very characteristic and not readily confused with any other form of malarial parasite commonly found in the peripheral blood. In some severe estivo-autumnal infections, showing many parasites of all stages in the peripheral blood, somewhat similar spheroidal or pre-segmenting forms may be found in considerable numbers, but, as will be seen by reference to Plate XVI., the character of the pigment in the estivo-autumnal parasite is very different, while such cases are very rare, and readily recognized on clinical grounds, being almost invariably of the pernicious type.

PLATE XV.



Cycle of Quartan Parasite.

Fig. 1. Very early non-pigmented form.
Figs. 2, 3, 4. Small quartan rings, with large chromatin masses and abundance of pigment.
Fig. 5. Turban-shaped ring, with subdivided chromatin.
Fig. 6. Subdivision of ring and of chromatin granules.
Fig. 7. Coarse quartan ring with central chromatin granules.
Fig. 8. Full-grown quartan parasite, with eccentric chromatin, hyaline body, and abundance of pigment.
Fig. 9. Extra-cellular reticulated body.
Figs. 10–13. Quartan presegmenting forms.
Fig. 14. Quartan rosette.
Fig. 15. Pigmented mononuclear leucocyte.

The quartan segmenting bodies are usually more abundant in the peripheral circulation than are rosettes of any other type, and are readily identified by the small number (six to twelve) and comparatively large size and geometrical arrangement of the spores. (Plate XV.)

The Estivo-autumnal Parasite. The following description applies to a group of organisms, which, according to the Italian school, comprises two or three varieties of malarial parasites. Waiving for the present the question of a plurality of species, the entire group will be described as one, and the grounds for their separation into two or more species will be considered later.

The earliest form of the estivo-autumnal parasite seen in the red cells is very similar to that of the tertian and quartan parasites, but is of slightly smaller size than either, and is often distinguishable from the tertian by the shrinkage of the cell, and from the quartan by its distinctly smaller dimensions. (Plate XVI., Fig. 1.) In fresh specimens the young ameboid body usually shows a low refractive index as compared with the tertian and quartan parasites. It is never pigmented. Associated with the intracellular spores there are frequently seen in the plasma small spheroidal bodies exhibiting an active rolling motion and occasional blunt projecting points (pseudopodia ?), and which, on becoming arrested by contact with the red cells, are found to be indistinguishable from compact intracellular bodies. The positive identification of these extracellular bodies, however, appears to the writer a very hazardous undertaking (cf. Ziemann, p. 49). In dried specimens stained by Nocht's method, however, the young extracellular parasite may be positively identified from the presence of a mass of chromatin. In the writer's specimens such extracellular bodies were very rarely encountered.

The Estivo-autumnal Signet-ring. At a very early period of its development the estivo-autumnal parasite assumes a very characteristic ring shape. Many of these rings early develop a thickening of one segment, and to these bodies of various sizes the term "signet-ring" very aptly applies. (Plate XVI., Figs. 5 to 7.)

It is to be noted that in some cases the rings fail to exhibit this thickening but remain of a *uniform but very fine calibre throughout.* (Plate XVI., Fig. 4.) The period during which the rings retain this uniform calibre has not been determined, but bodies of this type may be seen measuring at least 3μ in diameter. They nearly always present two nuclear bodies, lying at opposite poles or close together. Occasionally such rings are found to have unfolded, and to be stretched like a thread clear across the cell, the nuclei appearing at inconstant intervals.

In other cases no rings of this type are seen, all showing the thickening of the signet and a single nuclear body. In the majority of cases rings of both types are associated in variable numbers.

Multiple infection with the young rings is very common in the red cells of most cases of estivo-autumnal malaria, and, as a rule, its frequency is proportionate to the severity of the disease. In the peripheral blood three parasites are often found in the same red cell, and occasionally four, while in smears of the marrow of a fatal case, seen by the writer, infection of one cell with four rings was common, five parasites were occasionally seen in the same cell, and one slightly swollen red cell was encountered containing seven well-formed rings. These observations accord with the reports of Ziemann, who found often three and four parasites, and once as many as five in one cell.

It appears in the description of the *hemamœba immaculata*, which is said to sporulate without producing pigment, that most of the rosettes contain comparatively few spores, averaging from six to ten (Marchiafava, Bignami, Ziemann, Marchoux, Grassi and Feletti). The close resemblance to a non-pigmented rosette presented by some of these red cells harboring five, six, or seven young parasites is very striking. In the writer's specimens (see Plate XVI., Fig. 2) there could be no doubt as to the proper interpretation to be placed on these examples of multiple infection.

Multiple infection of the red cell appears in rather rare instances to lead to the development of a peculiar form of the young estivo-autumnal parasite on which Mannaberg bases his unique theory of the development of crescents. This body consists in the apparent union of two rings by a fusion of their nuclear bodies. (See Plate XVI., Fig. 3.) Mannaberg depicts all transition forms between these bodies and the fully developed crescents.

The signet-ring forms frequently reach a diameter of 4μ while still retaining the peculiar thickening of one segment, the thin geometrical bow, and a very distinct nuclear body staining with methylene blue and surrounded by a narrow achromatic zone. (Plate XVI., Fig. 6.)

Beyond this size, when persisting in the finger blood, the growth of the parasite produces an irregular body in which the outline of the ring becomes more or less obscure. The full development of the large signet-rings appears to require about twenty-four hours.

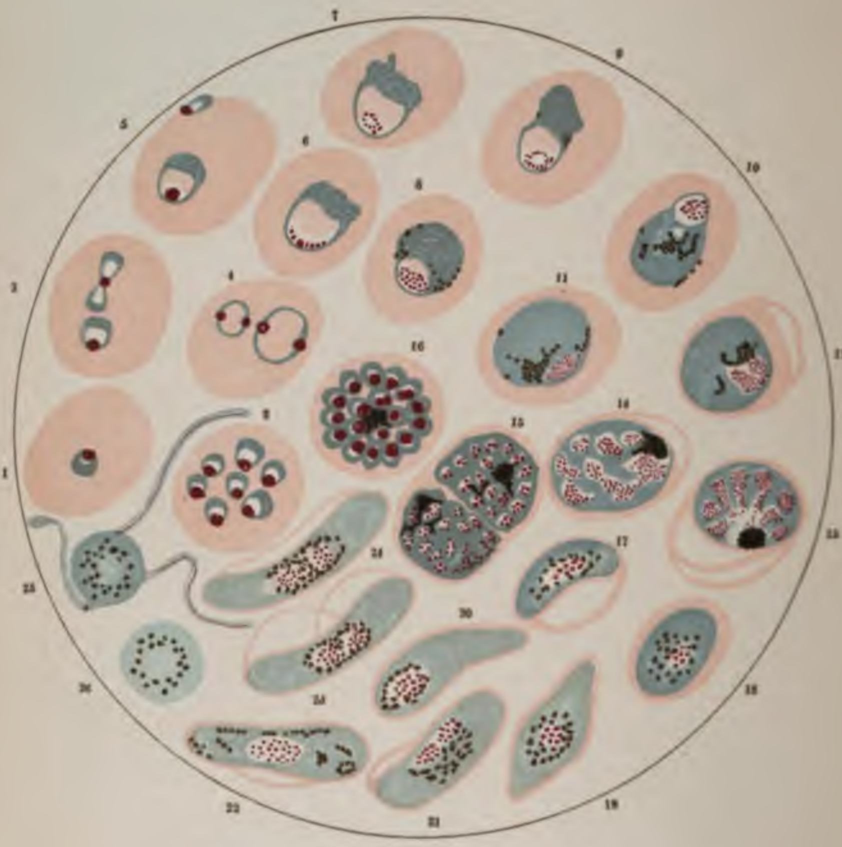
In the majority of cases the ring forms seen in the peripheral blood fail to show any trace of *pigment*, especially in the patients showing distinctly intermittent quotidian or tertian paroxysms. In a considerable number of instances, however, especially in very severe and fatal infections, the largest rings exhibit a very few minute pigment grains, usually associated with older pigmented forms.

The distinguishing features between the estivo-autumnal and the tertian rings have been enumerated under the description of the latter parasite.

Later Stages of the Estivo-autumnal Parasite. The later forms of the estivo-autumnal parasite are rather rarely seen in the peripheral circulation.

Most of the Italian writers speak of their occurrence in the finger blood as being very unusual, but not unknown. Saccharoff in two cases of estivo-autumnal infection saw many rosettes in the peripheral blood. Ziemann reports that in malignant tertian cases occurring in Italy he could follow in the blood the complete cycle, but that in cases occurring in Kamerun the later forms were not found in the finger blood. Plehn describes a variety of parasite which he believes is peculiar to hemoglobinuric fever, and of which the later forms are of very small size, but abundantly represented in the peripheral

PLATE XVI.



Cycles of Estivo-Autumnal Parasite.

Fig. 1. Very young form.
Fig. 2. Infection of one cell with seven young parasites. (Drawn from a marrow smear.)
Fig. 3. Triple infection. Two parasites joined by single chromatin mass.
Fig. 4. Double infection. Peculiar rings with two chromatin grains at opposite poles.
Fig. 5. Double infection. Small ring adherent to cell.
Figs. 6, 7. Signet-ring forms. Subdivision of chromatin.
Figs. 8, 9. Later ring forms, with subdivided chromatin and few pigment grains.
Figs. 10–12. Full-grown forms with finely subdivided chromatin and gradual concentration of pigment.
Figs. 13, 14. Stages of presegmenting forms, with concentrated eccentric pigment.
Fig. 15. Double infection with separate presegmenting bodies.
Fig. 16. Estivo-autumnal rosette.
Figs. 17, 18. Young crescent and ovoid.
Fig. 19. "Pulsating" crescent.
Figs. 20–22. Various forms of crescents.
Fig. 23. Two bows about single crescent.
Fig. 24. Fully developed crescent; two masses of chromatin; achromatic substance; double wreaths of pigment.
25. Diagrammatic flagellating body.
26. Extra-cellular sterile body.

blood. In five cases examined by the writer the entire developmental cycle of the estivo-autumnal parasite could be followed in the peripheral blood, and on the forms observed in these cases is based the present description of the later phases of this parasite.

After the ring has reached its full size (4μ, twenty-four hours +) the swollen segment begins to increase in bulk and to involve a larger portion of the circumference, yielding forms seen in Plate XVI., Figs. 8 and 9. Some of these forms closely resemble the turban-shaped rings of the tertian parasite (Plate XIII., Fig. 8), but are much smaller. A few fine pigment grains were usually found scattered along the periphery of the growing segment. Forms corresponding to the full-grown tertian parasite with homogeneous body are rarely seen in the peripheral blood. When encountered they are found to occupy three-fourths of the shrunken cell, stain homogeneously with methylene blue, and fail to exhibit a distinct nuclear body after methylene blue or hematoxylon.

Most of the larger forms of the parasite seen in the peripheral blood give evidence of approaching segmentation, exhibiting a distinctly reticular structure and a condensation of pigment into one or two clumps. (Plate XVI., Fig. 10.) In many of these bodies the original ring persists at one segment of the parasite, but appears to be of reduced size, and is sometimes subdivided by strands of protoplasm. The nuclear body at this period fails entirely to stain with methylene blue and is indistinct after hematoxylon, resembling in this respect the full-grown, homogeneous, tertian organism. The presence of a distinct achromatic spot adjoining the clump of pigment is very frequent in these forms, and this spot is found by Nocht's stain to be composed of chromatin granules.

The further development of the pre-segmenting forms is represented in Plate XVI., Figs. 11 to 15, two separate parasites in the same red cell being occasionally seen. In them the reticular structure becomes more distinct, the pigment is still further concentrated, and the subdivided nuclear bodies appear as small achromatic spots in the meshes of the reticulum and again stain distinctly with hematoxylon.

Estivo-autumnal rosettes appear in the peripheral blood of rare cases only and in moderate numbers, and exhibit a very uniform structure.

The pigment is grouped in a central granular clump, or, rarely, somewhat scattered. The spores seem to be arranged in two rows, but this appearance is probably an optical effect produced by the flattening of the more or less spheroidal body of the rosette, the spores originally lying in the central axis of the rosette falling, in the hardening process, within those lying on the periphery.

When admitting of accurate enumeration their numbers are found to vary between eighteen and twenty-one. The same number of spores was repeatedly counted in favorable specimens, made by the writer from the marrow of a fatal case. In sections of the tissues of fatal cases, however, the number of spores appears to vary between wider limits, *i. e.*, eight to twenty, but as the entire rosette need not always be included in the section the observations made in smears are the more reliable. A rim of hemoglobin invariably surrounds the rosette, and strands of hemoglobin are frequently found running between the spores for a variable distance, sometimes within the outer row.

These rosettes differ from the tertian segmenting forms in the smaller size of the body and shrunken appearance of the cell, and in the small size, but not in the number, of the spores.

The Changes in the Chromatin of the Estivo-autumnal Parasite.—In the young ring forms the early subdivision of the chromatin has been noted by Gautier, and in the writer's specimens was a prominent differential character from the tertain rings. A great variety of appearances is produced by the irregular subdivision and distribution of the chromatin in the young estivo-autumnal parasite, many of which have been sketched or described for the tertian rings. The grains are usually quite small and are sometimes apparently fused into a spindle-shaped mass, lying within the ring. Other peculiarities which may be noted are: a markedly unequal size of the grains, a widely separate position, a frequent concentration in the centre of the ring, and, very rarely, a complete absence of chromatin.

After twenty-four hours' growth the chromatin granules become more numerous and extremely minute, and are inclosed in a trace of the milky substance, as in the tertian parasite.

The changes in the chromatin in the pre-segmenting estivo-autumnal body are similar to those of the tertian parasite. In some of the writer's specimens the chromatin granules were found in radiating lines stretching from the parent mass to the new peripheral groups. In many specimens the peripheral groups of granules were well formed, while the central portions of the body contained many diffuse granules. The relative quantity of chromatin in some of these bodies appears surprisingly large. The spores in the mature rosette usually contain single compact grains of chromatin, which stain readily by methylene blue, but in some rosettes two large granules of chromatin are seen in a few spores, although the rosette seems ready to burst.

The double nuclei seen in many young estivo-autumnal rings may, perhaps, be referred to the incomplete fusion of the chromatin in the rosette.

Characters of the Pigment of Estivo-autumnal Parasites. When any considerable quantity of pigment gathers in the estivo-autumnal parasite it is usually found in one or two groups, but rarely is diffuse.

When the parasite has reached the full-grown, homogeneous stage the pigment is commonly found concentrated in a single compact mass. This early concentration of pigment is one of the chief features which distinguish the estivo-autumnal from the tertian parasite in the pre-segmenting stage. This fact has been fully emphasized by Gautier, and was very uniformly illustrated in the writer's cases.

The Plurality of Species in the Estivo-autumnal Group of Parasites. The probability that several species of parasites are concerned in the severe types of malarial fever prevailing in tropical countries, especially in the summer and autumn, has been chiefly maintained by the Italian school, who divide this group of parasites into two species: (1) the quotidian, and (2) the malignant tertian.

1. **The Quotidian Parasite.** The typical fever curve of this variety is rather rarely seen, more frequently in relapses than in initial seizures, while a postponement of paroxysms is usually observed, and a continuous fever is very common. The typical attack is short, the

fever lasting six to eight, rarely twelve hours, the temperature then falling to 37° C.

The descriptions of the morphology of this parasite unfortunately refer only to its appearance in fresh blood. During the rise of the temperature, the sweating stage, and the first hours of apyrexia, the blood is found to contain a variable number of red cells infected with one or more very actively motile or non-motile parasites of discoidal or ring shape. During the afebrile period the parasites increase in size, the ameboid motion diminishes or ceases, and fine pigment grains are deposited along the periphery of the organism. Later, in the larger forms, the pigment gathers in a single clump or heap of grains. During the entire development the infected red cell diminishes in size and presents a "brassy" color as a result of "acute necrosis" induced by the parasite. Rosettes are seldom encountered in the finger blood, segmentation occurring principally in the internal organs, as seen in the aspirated splenic blood. Rarely segmentation occurs before pigmentation, but usually the numerous round or oval spores are found grouped about a central pigment mass, the rosettes being much smaller than those of the quartan or mild tertian parasites.

Contrary to the rule in malignant tertian infections, the young parasites are found in the blood from the beginning of the paroxysms, and, except in very mild cases, there is no period in the cycle when the parasites are absent from the finger blood.

2. **The malignant tertian parasite** is distinguished on both clinical and morphological grounds. *Clinically* the typical paroxysm begins with a sharp elevation to about 40° C., the febrile period lasts twenty-four or thirty-six to forty hours, is marked by a *pseudocrisis* and *precritical* elevation, the fever describing in the three-hourly chart a very characteristic course which differs from that of the mild tertian paroxysms. A tendency toward various irregularities is common.

In the blood the parasites may be scarce or even entirely absent at the beginning of the paroxysm. At the height of the fever the red cells contain small, non-motile, ring, or disk-shaped bodies, or irregular, ameboid bodies, which begin to show pigmentation toward the approach of the afebrile period. Most of the parasites then disappear from the finger blood, and rosettes are rarely seen except in some very rich infections. The pre-segmenting forms are round or ovoid, one-fourth to one-half the size of the red cell, and the pigment is gathered in a single clump or in a mass of vibrating granules. The rosettes occupy about two-thirds of the red cell, and exhibit two rows of spores which usually number ten to twelve, rarely fifteen to sixteen. The infected cells are markedly shrunken and present a "brassy or golden" appearance.

The authors distinguish the malignant tertian parasite from the commoner or mild variety on the following features:

1. The malignant tertian parasite is smaller in all stages. 2. Its pigment is less abundant and often non-motile, while in the other the pigment is very abundant and always in vibratory motion. 3. The

rosettes are smaller, contain only ten to twelve (rarely sixteen) spores, and are rarely seen in the finger blood. 4. The infected cell is shrunken instead of being swollen, as with the mild tertian infection.

From the quotidian parasite the malignant tertian is distinguished on the following grounds:

1. The tertian ameba is, in corresponding stages, larger and less transparent than the quotidian.

2. In the malignant tertian parasite the ameboid movement is livelier, so that the resting discoidal forms are less frequent than with the quotidian parasite. The larger, pigmented, tertian forms also are often ameboid, this property persisting for twenty-four hours or longer.

3. The pigment in the tertian parasite is often vibratory, but never in the quotidian.

4. In the quotidian rosettes pigment is sometimes wanting.

5. The appearance in the finger blood of a new generation of tertian parasites is seen some hours after the beginning of the paroxysm, therefore much later than with the quotidian infection.

Marchiafava and Bignami admit that the similarity between the malignant tertian and the quotidian parasites is very great, and that the differential diagnosis is difficult, and possible only from the full-grown forms seen just before the paroxysm. They apparently do not feel quite certain that the quotidian and malignant tertian parasites are separate species, as is indicated by the following extract from their discussion on this point (*Sydenham Society Transactions*, p. 8):

"The remarkable points of resemblance between the quotidian and malignant tertian parasites make it very difficult to solve the question whether we have to do with different sorts of parasites in the strict sense, or with one and the same parasite which varies greatly in the time of its development—twenty-four to forty-eight hours—and there are all intermediate degrees. On this latter theory it becomes easy to ascribe the morphological differences to the varying length of the cycle. But various facts oppose this hypothesis. First, the clinical types of the quotidian and tertian are clearly distinct from each other, and have a certain stability which is maintained in relapses and recurrences. Second, we have never met with intermediate forms or transitional cases, although it is very difficult to interpret the irregular fevers. Granting that the question cannot, at present, be solved definitely, . . . we are inclined to adopt the view that the ameba of the quotidian and the ameba of the summer tertian are closely related varieties of one and the same parasite."

This view is not materially altered in the authors' last treatise (1900).

Mannaberg and Grassi and Feletti accept the views of Marchiafava and Bignami and describe a quotidian parasite, but do not furnish original observations tending to confirm their opinions. Thayer and Hewetson, Ziemann, and Gautier, all of whom have studied very minutely the estivo-autumnal parasites, the latter authors from extremely rich material in tropical regions, fail to find sufficient grounds for the subdivision of the group.

From the writer's study of cases at Montauk among soldiers arriving from Cuba in 1898, it was concluded that it is impossible from the observation of parasites in the peripheral blood to demonstrate the existence of a twenty-four-hour cycle, since the infecting broods are seldom compact. Quotidian paroxysms always seemed to be referable to infection with two broods of malignant tertian amebæ.

Two features were noted, however, which offered some hope of distinguishing a quotidian from the tertian parasite :

1. The exclusive presence in a few instances of rings with double nuclei and without the signet.

2. The appearance of large numbers of very small adult parasites in the peripheral blood.

Neither of these features proved sufficient for the positive identification of a quotidian parasite. The peculiar rings were usually associated with the ordinary signet-ring forms, and the rosettes seen in peripheral blood were identical in appearance with those found in marrow-smears from fatal cases of malignant tertian infection.

It, therefore, seems necessary to conclude for the present that a special quotidian parasite has not yet been demonstrated and probably does not exist. As stated by Ziemann, it seems likely that estivo-autumnal fever is caused by a single species of parasite whose developmental cycle usually requires forty-eight hours, but may possibly be completed in twenty-four, or, as in one of his cases, may be prolonged to seventy-two hours.

The Hemameba Immaculata. Grassi and Feletti, Marchiafava, Celli and Bignami, Guarnieri, Saccharoff, Marchoux, and Ziemann report cases in which rosettes were found in the blood or viscera which were free from pigment. Most of these authors, while admitting that the parasites may occasionally sporulate without producing pigment, are not inclined to regard the *hemameba immaculata* as a separate species. Grassi and Feletti claim to have observed in a bird exclusive infection with a variety of parasite which fails to produce pigment, and regard the appearance in the human subject of rosettes without pigment as evidence of infection by a distinct variety of parasite. Mannaberg also accepts this classification.

In the report of the examination of the viscera of this bird no mention is made of the presence or absence of pigment, and it is impossible to determine whether or not the infection had failed to produce pigment in the viscera as well as in the peripheral blood.

In all cases in which pigment-free rosettes have been found in the blood of human subjects there have been found the usual pigment deposits and pigmented rosettes in the viscera. That there is considerable variation in the quantity of pigment produced by the parasite in fatal cases is shown by the reports by Marchiafava and Bignami of fatal cases in which a microscopic examination was required to show the presence of very scanty deposits in the viscera.

Ziemann mentions that he has seen a pre-segmenting body of the benign tertian type which was entirely free from pigment.

The writer believes that some examples of multiple infection of red cells have been mistaken for pigment-free rosettes, but this explanation can hardly apply to the reports of many such rosettes in the cerebral capillaries. The majority of observers, including Marchiafava and Bignami, believe that there is no pigment-free variety of human malarial organism, but that the estivo-autumnal parasites may occasionally fail to elaborate pigment.

The Crescentic Bodies. On the fourth to sixth days of any but initial paroxysms of estivo-autumnal infection the peripheral blood may contain red cells infected by spheroidal, oval, elliptical, or small crescentic bodies which represent the early forms of the sexual cycle of the parasite. The relation of these forms to the young ameba is not clear, and it is not known whether the crescents develop directly from the spores or from some parent body, such as is seen with various coccidia, and *in which the young parasites are of crescentic form before their discharge from the mother cell.* The latter view is the more probably correct, being favored by analogy, but the earliest forms of crescents are very frequently spheroidal or elliptical. On the other hand, neither direct observation nor analogy favor the view that they spring directly from the ordinary ameboid parasites of the pyrogenous cycle, as claimed by Marchiafava, Celli, and Bignami.

Maurer describes the development of crescents (gametes), both from the differentiation of the small, ring-shaped, ameboid parasite, and from the merozoites of sporulating crescents. He identifies the early developing crescent on the following features: It first exhibits a ring form, but of coarse calibre, with one or two chromatin granules lying in the protoplasm of the parasite. It soon enters the red cell, where it loses the ring form and becomes compact, whereas the ameboid ring long remains on the outside of the cell. Within the cell it soon develops considerable pigment, absorbing the Hb and contracting and condensing the body of the cell without the formation of the striking changes in the cell described by Schuffner as *tüpfelung* (stippling). This breaking up of the cytoplasm of the red cell into coarse granules Maurer claims to be present only in cells infected by the ameboid parasite. In its further growth the gamete becomes enlarged, spheroidal, then kidney-shaped or crescentic.

Mannaberg regards the crescents as the syzygia developing from the union of two young parasites, and, while this view is lacking in support from the actual demonstration of two parasites uniting to form the crescent, and is entirely contrary to the recognized mode of origin of homologous forms in other protozoa, there are some morphological features which are somewhat in its favor. These are the appearance, frequently, of two groups of pigment, occasionally of two masses of chromatin, and the isolated observation of two halters with one crescent.

Appearing first as bodies somewhat shorter than the diameter of the red cell, through various changes in length, breadth, and shape (see Plate XVI.), the adult crescent is developed, appearing in the blood usually after the fifth to the seventh days of the paroxysm. The average adult crescent measures about 9μ to 12μ in length, by 2μ to 3μ in breadth, but in some cases very large or giant crescents have been observed, the writer having seen specimens measuring 20μ by 5μ. While the young crescent contracts and swells, altering its shape, the older forms are quiescent, and neither show true ameboid properties. The ends are either pointed or blunt, many old specimens appearing with swollen ends.

The *pigment* is in the form of coarse, golden-yellowish grains, rods, or possibly at times in rod-shaped crystals. It is the coarsest pigment elaborated by the parasite, and is always more abundant than in the ameboid bodies of equal size. It is arranged in a single central mass or circle, or as a double circle resembling a figure 8. Occasionally it is found in scattered groups, especially in very large crescents.

Although a membrane cannot be said to exist about the crescent, yet its outer border may be stained reddish by eosin, and it has, therefore, been supposed that a remnant of Hb surrounds the crescent on all sides. The membrane or thickened outer border of the red cell is closely applied about the convex side of the crescent, while across the concavity it stretches loosely like a halter. The writer has seen two of these halters spanning equal segments of the concavity of a single large crescent (Plate XVI., Fig. 23), while Marchiafava and Bignami have seen two adult crescents within a single cell.

In the centre of the crescent is a sharply marked, light-blue staining, or achromatic area of variable size, containing the chromatin and usually also the pigment. Marchiafava and Bignami describe the nucleus of the crescent as vesicular, but for reasons already stated the writer is unable to regard the nucleus as consisting of any other structure than the mass of chromatin granules, which can always be found in living crescents. In the young crescent these chromatin granules are of larger size than in the adult body, in which they become subdivided, and, when obscured by pigment, extremely difficult to detect. Sometimes there are two separate groups of chromatin granules. Marchiafava and Bignami expose most specimens containing crescents in a moist chamber for a few minutes, thereby causing the pigment to separate, the nucleus to swell, and the chromatin granules to be more apparent. With Nocht's method this expedient is seldom necessary.

Maurer describes certain features which he claims may distinguish the male (microgamete) from the female crescent (macrogamete). The male is short, thick, and kidney-shaped, or swollen at one end, densely staining, and its pigment is more often scattered. (Plate XVI., Figs. 17 to 23.) The female is more elongated, less densely staining, and exhibiting more compact pigment grouped about two or three centres.

Although crescents may show transverse segmentation and occasionally lateral budding, these changes are probably degenerative, as is also their vacuolation. Former views regarding their multiplication by various methods are now known to be erroneous. Maurer's recent description of sporulating crescents is quite unsatisfactory.

That the marrow is a special seat of the development of crescentic bodies is believed by Councilman, Bignami, and Bastianelli, who have found an excessive number of young forms in this tissue when they were scarce elsewhere.

The long persistence of crescents in the blood and their resistance to quinine are matters of common clinical observation. Yet Leukowicz, who describes several varieties of crescents, denies that they are any more refractory to quinine than are the ameboid forms.

Flagellate Bodies. When blood containing crescents is allowed to stand in the air or under a cover-glass for a few moments some of the crescents slowly assume the spheroidal form, active vibratory oscillations of the pigment granules begin, and soon, from one or more points, pseudopodia shoot out with active lashing movements. These flagella continue their movements for some time, changing their position actively, their shape slowly, while some may be seen to break off from the body and swim off through the plasma. The formation of flagellate bodies represents the second stage in the sexual cycle, probably never occurring in the human body.

These flagellate bodies are found, on staining, to be composed of a spheroidal, pigmented mass, usually surrounded by a remnant of the red cell, of two to four flagella of variable type, of one or more lateral buds, and of chromatin, which has now undergone a remarkable transformation. The bulk of chromatin is apparently much increased, and most of it is usually found in the form of long, slender threads within the flagella. Occasionally short threads of chromatin may be found within the sphere. The flagella are composed of an outer protoplasmic covering, their ends are blunt or pointed, their borders even or bulbous, and while most of them contain chromatin threads some are entirely lacking in this constituent.

Not all crescents develop flagella. Some remain in the semilunar form, exhibit slow, undulatory movements, and tend to stain more deeply with methylene blue. It is believed that these crescents are penetrated by a motile flagellum, after which they become actively locomobile, and it is further supposed that these motile fertilized crescents or "*vermiculi*" are capable of penetrating the wall of the mosquito's stomach, and there developing the encysted bodies, which are the next stage of the bisexual growth of the parasite.

The evidence on which this relation of two varieties of crescents is indicated is largely derived from analogous phenomena, known to occur among various coccidia. Simond and Siedlecki, in the coccidia, have seen the fertilization of one crescentic body by the motile flagellum of another, and MacCallum, examining the parasites of crows, has seen the entrance of a flagellum into a crescent with the development of a motile vermiculus. (See Solley and Carter.) The same evidence indicates that the flagella are motile chromosomes of a karyokinetic nucleus, and that their function is that of a male fertilizing element. It thus appears that the crescents and their flagellated derivatives constitute a developmental series destined to perpetuate the species in the body of the mosquito.

Tertian flagellate bodies develop from the full-grown tertian parasites of long-established infections in much the same way as from crescents. Of full-grown tertian parasites three varieties must be separated: (1) bodies which are destined to segment, and which the writer believes are often produced by conjugation; (2) large hyaline forms which develop flagella; (3) large hyaline forms with little chromatin which become fertilized by detached flagella. This last form has often been described in shed blood, and the writer has observed its fertilization in stained specimens of flagellate bodies.

In the tertian parasite which is developing flagella, the chromatin increases in quantity, becomes arranged in a basket network, breaks up into several coarse filaments which may be found protruding

PLATE XVII.

Fig. 1.

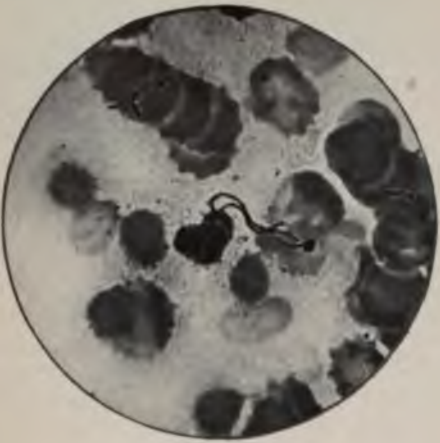


Fig. 2.

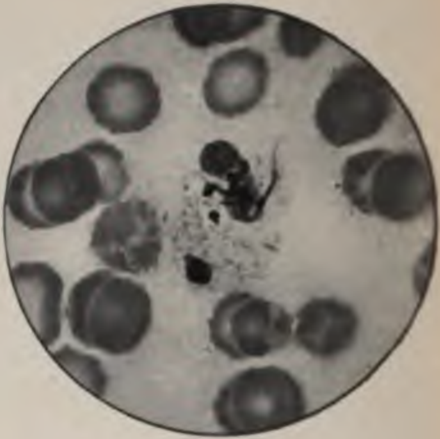
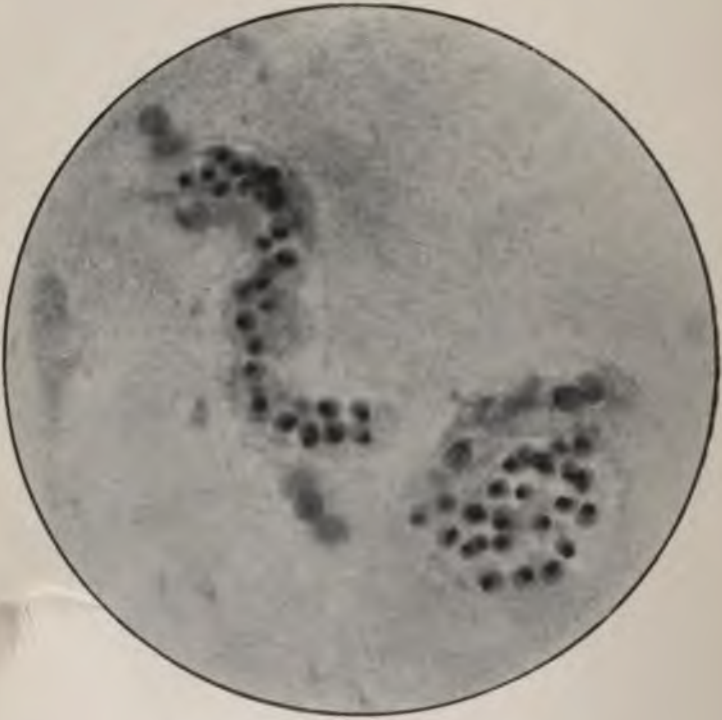


Fig. 3.



Flagellating Malarial Parasites.

Cerebral Cortex in Pernicious Malaria.

Fig. 1. Tertian flagellate body.

Fig. 2. Tertian flagellate body plunged into a red cell containing a macrogamete.

Fig. 3. Estivo-autumnal parasites in cerebral capillaries.

Photographs by Dr. B. H. Buxton, from specimens prepared by the author and stained by Nocht's method.

from the edge of the sphere, and, finally, these filaments are discharged in the form of flagella, of which there are usually six to each parasite.

Quartan parasites develop flagellate bodies very similar in appearance to those of the estivo-autumnal type.

III. THE DEVELOPMENT OF THE MALARIAL PARASITE IN THE MOSQUITO.

It is principally owing to the labors of Ross, at Manson's suggestion, that the life cycle of the parasite in the mosquito has been elucidated. Ross followed the development of the *proteosoma* of Labbe in the tissues of mosquitoes which had fed upon the blood of birds containing this malarial parasite. He traced the development of an encysted body in the wall of the intestine, and the formation therein of large numbers of so-called germinal rods. Finally, when the germinal rods were found abundantly in the salivary glands, the complete cycle of the parasite in the mosquito, and its natural mode of access to the human body were made clear. The demonstration was rendered doubly positive when Bignami succeeded in transferring the estivo-autumnal infection from one human being to another by passing it through *Anopheles claviger*, a species of mosquito found in the Roman Campagna. Later, tertian infection was transferred in the same manner.

The details of the development in the mosquito have been worked out principally by Grassi, Bignami, and Bastianelli, while much valuable information regarding the few dangerous varieties of mosquitoes, their habits, and the means of identifying and destroying them have been contributed by Ross, Nuttall, the Italian writers, and by the Jenner School of Tropical Medicine.

Developmental Forms. Two days after the mosquito has fed upon blood containing crescents there are to be found in the intestinal submucosa spindle-shaped, oval, or spheroidal bodies, resembling the spindle-shaped bodies of the blood. They contain a single nucleus, scattered pigment, and their protoplasm appears vacuolated. The actual development of these bodies from the crescents and the penetration of fertilized crescents into the intestinal wall have not been demonstrated.

By the third to fourth days the bodies are much enlarged, distinctly encapsulated, their protoplasm is reticulated, and their pigment has largely disappeared.

By the fifth to the sixth days the encysted bodies have greatly increased in size, measuring up to 70μ in diameter, and project into the celomic cavity. They are now found to contain numerous nuclei and globules resembling fat.

By the seventh day fully formed germinal rods are found in the cyst in enormous numbers. These rods average about 14μ in length, their ends are pointed, and at the centre of each is a granule of chromatin. They are arranged in radiating or undulating masses about numerous centres.

After the seventh day broken capsules are found which have discharged their rods into the celom, from which region they make their way into the salivary glands.

In addition to encysted bodies which develop rods, some contain "brownish bodies" of various sizes, the nature of which is not known.

Very similar phases have been described for the development of the tertian parasite, while Bignami and Bastianelli have recently succeeded in tracing the similar phases of the quartan parasite.

IV. CONJUGATION OF MALARIAL PARASITES.

In 1897 the writer's attention was attracted by a specimen of blood from a rich tertian infection in which there were very numerous twin parasites of a younger brood, while all the older parasites were single. A remarkable specimen of this type was secured at Montauk (in 1898) in which both broods were very compact and very numerous, and nearly all the younger parasites were twinned, while all the older forms were single. Extreme length and variety of ameboid processes also characterized the younger parasites in both specimens. Later the writer[2] was able to follow such a case through one complete cycle, and found that the young twins were succeeded by full-grown single parasites of larger size. Finally, the application of Nocht's stain furnished convincing pictures of all stages of union, first of the bodies, later of the nuclei of the twin parasites, and left no room for doubt that under some conditions malarial parasites undergo a form of conjugation.

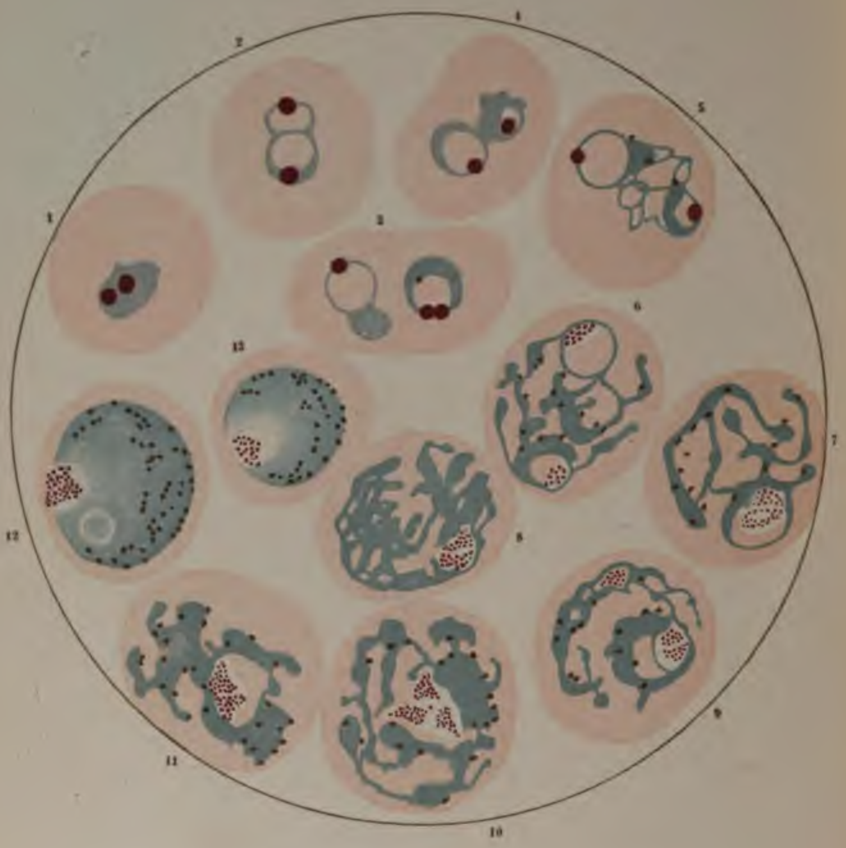
The phases of this process are sketched in Plate XVIII.

Usually the members of the conjugating pair presented distinct differences in appearance. One was a thick, compact parasite with abundant cytoplasm, one large and sometimes one smaller granule of chromatin, and was seldom seen in the ring form. The other was a ring-shaped parasite of delicate contour, of larger diameter, and with rather less chromatin.

The separate development of each of these parasites was followed in these cases up to the large, spheroidal bodies. The compact parasite usually retained its compact form throughout, producing a parasite resembling in size the quartan forms, and staining very densely with methylene blue. In some instances it developed six or eight separate masses of chromatin granules, but the writer could not be certain that it sporulated. The delicate rings, developing singly, produced medium-sized, pale-staining parasites of full-grown appearance, but beyond this stage it was not followed. The conjugating parasites produced large segmenting forms with sixteen to twenty spores.

The extent and significance of this form of conjugation it is difficult to determine. In the cases in which it can be profitably studied, parasites are usually very abundant, and in most cases few indications of the process can be detected. It is, therefore, probably not essential to sporulation, and when parasites are scanty the chances of finding typical examples of conjugating pairs are greatly reduced, but the peripheral blood may not be a complete index of the processes in the internal viscera. The large size of the sporulating forms developed from conjugating pairs suggests that this process is intended to especially favor the multiplication of the species in the human host. It seems probable that conjugation occurs in the first genera-

PLATE XVIII.



Conjugating Cycle of Tertian Malarial Parasite.

Fig. 1. Single compact body with double chromatin masses.

Fig. 2. Conjugating rings of unequal size.

Fig. 3. Double infection with a coarse ring, double chromatin granules, and a thin ring form.

Figs. 4, 5. Early stages of conjugation of a thin ring and a compact body.

Fig. 6. Early ameboid figures of conjugating rings.

Fig. 7. Double nuclei in ameboid parasite.

Fig. 8. Union of nuclei, and subsidence of ameboid motion in older conjugating parasites.

Figs. 9, 10. Stages of union of bodies and of three chromatin masses, of two conjugating parasites.

Fig. 11. Complete union of bodies and nuclei.

Figs. 12, 13. Comparative sizes of full-grown forms developed with and without conjugation.

tions of the infection and becomes less frequent as the disease progresses, the infection in the human host thereby tending to limit itself.

Several sources of error in the interpretation of these appearances have to be considered. The presence of two masses of chromatin has been noted in single young parasites. Yet the existence of the bodies of two parasites can usually be determined with certainty, while some conjugating forms contain three large masses of chromatin granules, *i. e.*, when one of the conjugating forms supplies an extra granule. The conjugating forms must, therefore, be identified from the presence of the bodies as well as the chromatin masses of two parasites.

The death and extrusion of one or of both of the twin parasites cannot account for the absence of older, twinned forms, since no traces of such extrusion were encountered. Twin parasites sometimes proceed to full development without conjugating, but this event is comparatively rare.

The existence of these three types of full-grown tertian parasites naturally suggests that the single, compact form develops into the microgametocyte or male flagellating body, the single, ring-shaped form developing into the macrogametocyte or female form, and the conjugating form into the sporulating body.

The above observations regarding the existence of three varieties of full-grown tertian parasites have recently been verified by Argutinsky.

The further attempt to trace the younger forms of male and female tertian parasites is beset with great uncertainties. The author finds that it is usually possible to detect the gradual differentiation of the sexes after the infection has progressed through several paroxysms. In these prolonged cases which have been allowed to run on without quinine the appearance of the parasites is strikingly altered. Small, compact parasites, without rings, become abundant, but the thin ring forms nevertheless persist; forms resembling quartan parasites appear, and in some of these four to six groups of chromatin granules appear in the body. These latter forms seem to be the ones which flagellate, and the fact that they appear abundantly only in the later stages of infections accounts for the frequent failure to secure flagellating bodies when the blood is drawn early in the disease.

V. THE OCCURRENCE OF PARASITES IN THE CIRCULATION.

In all well-marked initial attacks of malarial fever the parasite can be found in the blood if examined within eighteen hours after the chill. The writer found this rule to hold even in patients who were taking large doses of quinine, but in one case examined twenty-four hours after the chill parasites could not be found during a search of two hours through stained specimens.

In the great majority of cinchonized cases of acute estivo-autumnal fever, parasites can be found within a week after the beginning of the paroxysm, while crescents may persist for two or three weeks or more. In cases treated by quinine the writer found the ameboid parasites to largely disappear from the blood on the third day. If no crescents appear, the blood then becomes entirely free of parasites, but usually crescents enter the circulation on or after the fourth day and the examination of the blood still continues positive.

On the other hand, with very mild or with cinchonized cases a prolonged search is sometimes required for the detection of parasites.

In a few relapses of estivo-autumnal infection, examined during the chill, the writer had to search thirty-five to fifty minutes before discovering a single parasite, and in several other cases only young crescents could be found, while if the examination was delayed for eighteen to tweny-four hours it was often necessary to search for two hours or more. With Nocht's stain this labor would probably have been reduced.

In cases that have not taken quinine, the parasites are usually so abundant in well-marked infections that no difficulty whatever is experienced in determining their presence.

Yet the well-known tendency of the estivo-autumnal parasite to retire from the peripheral blood during the sporulating stage may leave the finger blood comparatively free from organisms at certain periods of the attack. In the tropics Van der Scheer noted that if the blood is examined during the sporulating stage no parasites may be found, and that they may appear, in scanty numbers only, after some hours' growth. Marchiafava and Bignami also emphasize the fact that a negative result may frequently be obtained at the beginning of an attack. Likewise in mild tertian infections initial paroxysms occur in which very few parasites are present in the blood, and in some instances *they have been reported* as absent altogether. The same fact has been observed in connection with experimental infections.

Nevertheless, the writer believes that in acute malarial fever, even when quinine has been administered, parasites can always be found, *if the blood is examined with care and persistence within eighteen to twenty-four hours after the chill.*

In the majority of treated cases the usual phases of development of the parasite may be found in diminishing numbers.

Although Golgi holds that the number of parasites in the peripheral blood is no reliable indication of their total numbers in the body, yet the severer cases nearly always show the larger numbers, while in mild cases parasites may be very scarce.

In conditions of coma the blood may contain either rings or crescents, or both, or tertian parasites. When coma arises in the course of the active sporulating cycle, it is almost invariably fatal, and the presence of many ameboid parasites in the blood of such cases is an extremely unfavorable sign. In most cases of malarial coma, especially those of very abrupt onset, or with symptoms of meningitis or of localized cortical irritation, crescents only are found in the blood and recovery usually follows.

In some cases of coma the writer had to search one and two hours before finding a single parasite, and has encountered some cases of coma and convulsions, in subjects believed to be suffering from malaria, in which no parasites could be found in the blood.

The number of parasites to be found in the blood, when they are extensively massed in the gastro-intestinal mucous membrane, heart muscle, brain, etc., has usually been considerable. Yet the writer encountered a few cases of acute estivo-autumnal infection marked by violent hematemesis, in which very few parasites were discoverable in the blood. In one case of malarial hematemesis no parasites could be found during the first three days of the paroxysm, but on the fourth day numerous young crescents appeared.

Fatal Malaria Without Parasites in the Peripheral Blood. The question whether fatal malaria exists without the presence of demonstrable parasites in the blood has not yet reached complete solution. There can be no doubt, however, that in acute paroxysms, fatal on the fourth or fifth day or later, the energetic use of quinine may rid the blood of all ameboid bodies, although the patient dies from the attack. When the blood is not examined until the third day of such attacks the result may be negative.

Marchiafava and Bignami have described peculiar fatal cases of estivo-autumnal infection occurring in debilitated subjects, during very hot weather, in which few or no parasites were found in the blood and very scanty deposits of pigment were present in the viscera. The writer has reported a similar case, in which, however, quinine had been administered for some days before the examination of the blood. In spite of these apparent exceptions to the rule, the writer believes, with Marchiafava and Bignami, *that fatal acute malaria does not exist without the presence of parasites in the blood, at least at some time during the paroxysm.*

Remittent Malarial Fever. *In remittent malarial fever,* persisting longer than seven days, the writer, in thirty selected cases, found only estivo-autumnal rings in eight; crescents alone in eleven; both rings and crescents in one; tertian parasites alone in one; double infection in one; and no parasites in eight. Initial estivo-autumnal attacks often begin with remittent pyrexia, and some relapses may show a temperature which requires three days to reach its acme and three or four to decline. These cases do not properly fall in the class of remittent malarial fever, but they probably illustrate the self-limiting tendency of malarial infection.

In chronic malaria, organisms, principally crescents, are usually to be found in the blood when there is fever, but are commonly absent during afebrile periods. In one case the writer found crescents in the blood four weeks after the last chill, quinine having been constantly administered.

Long after parasites have disappeared, *scanty pigmented leucocytes* may be encountered in the blood, but the situations in which their discovery proves of value in diagnosis are comparatively rare. *Their detection is more often of value in cinchonized cases of acute fever, in which parasites have disappeared.* Pigmented leucocytes are most abundant shortly after the chill in relapses of well-established estivo-autumnal infection.

Since Marchiafava and Bignami admit that in chronic malaria properly treated mild paroxysms may fail to reduce the number of red cells, and since quinine may reduce or destroy the scanty number of parasites ordinarily thrown into the peripheral blood, *it is neces-*

sary to admit that paroxysms may occur in chronic malaria without diminution of red cells and without parasites in the blood.

Yet such cases must be *excessively rare* and by no means open the door to the indiscriminate diagnosis of malaria in obscure fibricula without anemia or parasites in the blood.

Typhoid Fever and Malaria.

The effect of intercurrent typhoid infection upon the blood in malaria is very distinct. The concentration which results in typhoid fever becomes at once apparent, the blood drop appears distinctly less fluid, and the Hb and proportion of red cells rise.

During the first week of the disease sporulation of parasites frequently continues and organisms may be found in the blood as usual. Illustrations of this coincidence of active malaria in the first week of typhoid fever are rather numerous in recent literature. *After the first week, when the typhoid fever has become fully established, active sporulation of malarial parasites is extremely rare.*

Until recently there was apparently but one case, reported by W. Gilman Thompson, in which active sporulation of the malarial parasite was shown to have occurred at the height of the typhoid infection.

Of many others, collected by Lyon, most prove to be examples of malarial paroxysms occurring in the first few days of, or during convalescence from, typhoid fever. So rare is the coincidence of parasites in the blood during the course of typhoid fever that neither Marchiafava, Bignami, Bacelli, Dock, or many others, have ever seen a single case. In 159 cases of typhoid fever, nearly all occurring in actively malarious subjects, at Montauk, the writer could find no instance in which parasites were present in the blood during the second to the fourth weeks of the disease, although several early or convalescent cases suffered active malarial attacks with parasites, always tertian, in the blood. Cases of quartan infection associated with typhoid fever are reported by Thayer and by Craig.

VI. THE ACTION OF QUININE ON THE MALARIAL PARASITE.

The action of quinine may be followed in the blood of patients treated by this agent, or in fresh specimens to which weak solutions of the drug are added.

By the latter method, when a solution of quinine sulphate, 1 to 1500, is allowed to mix with blood under a cover-glass, it can be seen that most parasites soon cease their ameboid movements, although vibratory motion of pigment grains may persist for many hours. Most of the parasites contract and remain motionless, but, according to Monacho and Panichi, the half-grown parasites often leave the red cell and are englobed by phagocytes, while the younger and older forms do not emigrate and may long show ameboid movements. These authors explain from these facts the inefficacy of quinine in any other than the apyretic stage.

In the blood of patients treated by quinine the parasites often disappear rapidly without exhibiting morphological changes.

According to Golgi and Mannaberg, the administration of quinine soon limits the ameboid activity of all forms and induces fragmentation and hydropic changes of many. Golgi describes as "quinine forms" certain rounded, immobile bodies with clumped pigment and sharp, irregular outline, and deformed segmenting bodies deficient in the number of spores. Romanowsky and Mannaberg find that many of these segments are lacking in chromatin. In cinchonized cases the writer has often encountered half-grown forms numerously subdivided and deficient in chromatin.

Most observers agree that quinine is active only on the young, ameboid forms, and hence should be administered a few hours before the chill.

VII. THE MALARIAL ANEMIA.

There are few conditions which lead so rapidly to such extreme oligocythemia as does acute malarial infection. Kelsch, who in 1875 contributed an exhaustive study of malarial anemia, found that an acute initial attack lasting three weeks might reduce the red cells to 1,000,000 or even to 500,000. In robust subjects suffering initial attacks the loss on the first day sometimes reached 1,000,000 red cells, or during the first four days 2,000,000. Dionisi observed a reduction of 500,000 cells in twelve hours. In relapses the seizure did not cause so great a loss of cells as in first attacks. In prolonged cases it was often possible to distinguish three periods in the progress of the anemia: (1) During the first three to four days the loss of red cells was very rapid (1,500,000 to 2,000,000). (2) During the afebrile interval and during the relapse the fall was continuous, but much less rapid. The minimum of red cells varied with the character of the infection, being lower with estivo-autumnal cases, frequently falling below 2,000,000, seldom below 1,000,000. (3) When the patient became very anemic, relapses reduced the red cells very much less than before, but the active regeneration of cells caused their numbers to oscillate. So energetic may this reproduction of cells become that even an increase, instead of decrease, may be observed directly after an attack in anemic subjects.

Initial attacks of pernicious type cause very marked loss of cells, but in relapsing pernicious cases, with severe established anemia, it is not uncommon to find the severest general symptoms of the paroxysm unaccompanied by demonstrable loss of red cells.

Marchiafava and Bignami find that there may be destruction of red cells without fever in larval malaria.

In *chronic malaria* the prolonged infection, poor treatment, and bad hygiene commonly reduce the red cells below 2,000,000, but some extreme cases show below 1,000,000, Kelsch having one case with only 583,000. On the other hand, in favorable subjects, when attacks occur at intervals of one month or longer, and are promptly stopped by quinine, there may be no reduction of red cells (Marchiafava, Bignami). The writer can find no evidence on which to extend this exception to the Hb as well.

In afebrile periods, both between relapses and after subsidence of the infection, the anemia may still progress. In fact, the writer found this further progress of post-febrile anemia to be the rule in all very severe cases, and sometimes it proved fatal. Dionisi observed a fall from 3,200,000 to 2,300,000 of red cells during the first six days of apyrexia, in spite of the use of iron. The regeneration of the blood after benign tertian infections usually begins at once, but after all pernicious attacks both the response to iron and the progress of regeneration are slower.

Morphology of Red Cells. In cases of moderate severity the usual changes in the red cells of secondary anemia are present. Even in the early stages of anemia, polychromasia and granular degeneration of red cells are sometimes to be noted, while these changes increase steadily as the anemia progresses.

The grave post-malarial anemias present some very interesting forms. Marchiafava and Bignami describe three types, based upon the changes in the blood and marrow :

1. The red cells exhibit the characters of grave secondary anemia with extreme loss of Hb, but no nucleated red cells are present. The marrow shows simple atrophy of lymphoid cells and of clusters of nucleated red cells.

2. With the same condition of the blood the usual number of nucleated red cells may be found, and there is moderate hyperplasia, of physiological character, of the lymphoid marrow.

3. The red cells exhibit the characters of progressive pernicious anemia, with high Hb-index, and with megalocytes and megaloblasts. The marrow suffers typical megaloblastic degeneration.

The writer's study of blood and marrow in fatal cases supports this division of post-malarial anemias. While the ordinary features of grave secondary anemia were usually found, there were in the writer's Montauk series no less than nineteen cases in which the changes of the progressive pernicious type had been established in a period not longer than ten weeks. In one of these the parasites appeared to have been massed principally in the bone-marrow, where their numbers were enormous and instances of multiple infection of cells were excessively numerous. There can be no doubt that the tendency of the estivo-autumnal parasite to be massed in the bone-marrow, in both ameboid (the writer) and crescentic phases (Councilman, Bastianelli, Bignami), and the excessive demands on red-cell production arising in the disease, render pernicious malaria an extremely favorable condition for this disturbance of the structure of the marrow and the development of specific megaloblastic changes.

In nearly all cases of grave malarial anemia polychromasia is a marked feature, and granular degeneration becomes so prominent that Plehn and others have even described as a peculiar form of the parasite those collections of bluish staining granules which appear in many badly degenerated cells. This form of degeneration was very pronounced in a fatal case of benign tertian infection reported by the writer, in which there was also a remarkable grade of hydremia, and, in the kidneys, the lesions of hemoglobinuric fever.

Besides the lesions referable to anemia, or to the toxemia of paludism, the infected red cells suffer a peculiar series of changes resulting from the presence of the parasite. Changes in the size of the cell are nearly constant. The tertian parasite, almost from the first, causes swelling of the cell and progressive loss of hemoglobin, as indicated by increasing pallor. When harboring two or three large tertian parasites the red cell may become enormously distended.

When infected by the quartan or any form of estivo-autumnal ameba, the red cell usually shrinks and takes on a peculiar, opaque, "brassy" color. This change begins at the moment of infection by the young ring and becomes more marked with the growth of the parasite. Marchiafava and Bignami suggest for this condition the appropriate term "erythropyknosis." These authors believe that the pyknosis results in the death of both cell and parasite.

In many cells infected by any form of parasite, especially the estivo-autumnal, the hemoglobin collects in a dense layer about the parasite, and achromatic clefts often form in the red cell. (Plate XVI., Fig. 13.)

Fragmentation of the red cell is a rather infrequent result of infection by the parasite.

Bignami has described an increased cohesiveness of the infected red cells in estivo-autumnal malaria, and explains thereby the tendency of infected cells to gather in small visceral capillaries.

Pathogenesis of Malarial Anemia. In the pathogenesis of malarial anemia it is necessary to consider the action of several factors. Each infecting parasite probably destroys more or less completely the harboring cell, and a certain part of the loss of red cells must be referred to this source. Yet Dionisi has shown that there is no very close relation between the number of parasites present in peripheral blood and the destruction of red cells which results from a paroxysm.

Moreover, the plasmodium does not immediately enter the cell, but remains for a time merely attached to its surface, and since quinine may cause these extraglobular parasites to abandon the corpuscle, as described by Marchiafava and Bignami, it is probable that in many paroxysms, properly treated, the destruction of cells resulting from direct consumption by parasites is very slight.

Many factors indicate that the post-critical anemia is principally referable to globulicidal action of the serum, dependent upon the presence of a malarial toxin. Some of these facts are the disproportion between the anemia and the number of parasites present ; the steady diminution in the loss of cells in the second and third, as compared with the first paroxysm ; the further progress of anemia in many cases after parasites have disappeared from the blood (Dionisi, the writer) or during intervals between paroxysms (Kelsch); the occurrence of hemoglobinuric malarial fever, and the increased resistance of cells demonstrated by Viola during the progress of malarial infection. The writer has pointed out at some length that the chief bulk of pigment deposited in viscera is derived from the globulicidal action of the plasma and not from the vegetative functions of the parasite.

Increased production of red cells greatly alters the progress of malarial anemia, being partly accountable for the smaller losses observed after secondary paroxysms, and preserving intact or even increasing the numbers of cells after paroxysms in very anemic individuals.

VIII. THE LEUCOCYTOSIS OF MALARIA.

Most observers have found very little change in the numbers of leucocytes in the finger blood during acute malarial attacks of average severity. This absence of leucocytosis with a rapidly rising temperature may be found of considerable corroborative value in the diagnosis of malarial fever.

A slight leucocytosis at the beginning of the paroxysm has been noted in some cases by Kelsch, Billings, Vincent, and others, but the numbers usually remain below 10,000, while the percentage of polynuclear cells is increased. Vincent finds that quinine tends to increase the polynuclear leucocytes throughout the entire paroxysm. With the falling temperature and during apyrexia the leucocytes are usually distinctly diminished (2000 to 4000), especially the polynuclear forms, giving a relative lymphocytosis.

Except during the three to four hours immediately following the chill, therefore, malarial blood usually shows a diminished number of leucocytes and a distinct relative lymphocytosis. The lymphocytes, small and large, may sometimes become quite numerous, especially in well-established cases. This fact accords with the increased cellular activity of the lymphoid tissues shown by microscopic examination of the viscera.

Marchiafava and Bignami refer the lymphocytosis to a specific chemotactic action, but it seems partly referable to simple mechanical factors.

The percentage of polynuclear cells at the beginning of the attack is usually high. Billings found over 80 per cent. in some cases. Later these forms diminish and usually fall below 60 per cent. In pernicious attacks Bastianelli found below 40 per cent. of polynuclear cells.

Bastianelli refers the loss of polynuclear leucocytes to the increased phagocytic activity of these cells. Vincent noted a periodical decrease in the number of large mononuclear cells which he refers to the same process.

In *the severer æstivo-autumnal paroxysms* many observers have noted a distinct leucocytosis (Kelsch, Babes and Georghiu, Ziemann, Burot and Legrand). Kelsch found that the leucocytosis of pernicious malarial attacks often consists in marked lymphocytosis, which the writer also has observed. Bastianelli and Bignami show that in addition to various inflammatory complications leucocytosis in pernicious malaria may result from rapidly progressive anemia. They find it to be of frequent occurrence in hemoglobinuric fever, and in cases attended with severe diarrhea. Plehn, however, found no leucocytosis in most of his cases of hemoglobinuria. The extent of the leucocytosis varies between 10,000 and 35,000 cells, the latter number having been observed by Kelsch shortly before death in a comatose patient. Usually the leucocytosis in pernicious attacks is much lower, and many attacks fail to cause any distinct increase.

The presence of eosinophile cells may be noted in most cases of malarial fever, and these cells are usually increased in number during afebrile periods. Grawitz rightly regards this feature as of diagnostic importance, as in most diseases likely to be confused with malaria eosinophile cells are long absent or scarce. Bastianelli and Bignami found that eosinophile cells diminish during the paroxysm and increase during apyrexia, while the blood is regenerating. In two cases of pernicious malaria, with many parasites, they found many mononuclear leucocytes and a very few *eosinophile myelocytes*, similar to those seen in myelogenous leukemia. Neutrophile myelocytes are often present in small numbers.

In chronic malaria Kelsch found the leucocytes usually subnormal in numbers, but in one of thirty-three cases there was transient leucocytosis.

Pigmented leucocytes are seen in the majority of cases, most abundantly in the severe and long-established fevers. They are found in nearly all fatal cases, but the writer found them most abundant in a case which recovered. It appears that the pigmented leucocytes are more closely related to the severity of the antecedent paroxysms than to the extent of the pigment deposits in the viscera.

They are most abundant during and shortly after the febrile period, but are often found in afebrile cases and after parasites have disappeared from the blood. The phagocytic cells seen in the blood include mononuclear and polynuclear leucocytes and endothelial cells. The large and small mononuclear cells are most often found to contain pigment or parasites, but in a few cases, for reasons not clear, large numbers of polynuclear leucocytes are found harboring rosettes, other forms of parasites, and pigment. In a few cases very large endothelial macrophages may be found in the blood, containing parasites in all stages of degeneration.

The objects englobed by phagocytes, as seen in the circulation, include: (1) Parasites, free or enclosed in red cells. (2) Pigment elaborated by parasites, usually in small clumps, sometimes in large masses. (3) Hematoidin derived from the destruction of red cells. (4) Hemosiderin derived from the detritus of red cells. (5) Intact or broken red cells. (6) Other leucocytes. Crystalline pigment is often seen in leucocytes in sections of tissues, but never in the circulating blood during life.

The degenerative changes in phagocytic leucocytes mentioned by Bastianelli and Bignami, including vacuolation and diminished staining capacity of nuclei, have been noted by the writer in many severe cases. The number of vacuolated leucocytes found in the blood is considerable and sometimes very large. Fatty degeneration the writer has been unable to demonstrate.

From a comparison of the phenomena of phagocytosis in the circulating blood with those seen in the viscera of fatal cases, it is evident that the phagocytic function of the leucocytes in malaria is very subordinate to that of the visceral phagocytes in all but a few exceptional cases.

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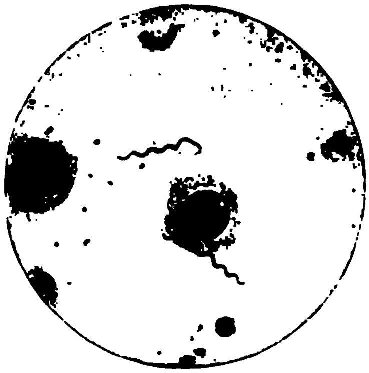
CHAPTER XXIV.

RELAPSING FEVER.

Morphology of the Parasite. The parasite of relapsing fever, discovered in the fresh blood by Obermeier in 1873, is a spirillum about 16μ to 40μ in length, being subject to considerable variations in size. It is very thin, sharply contoured, structureless, and resembles a curled fibrin-fibril. Soudakiewitch described irregularly contoured spirilla and some with a granular swelling at one end which he regarded as a spore. Several parasites may be twisted together, and occasionally considerable numbers are found in a granular-looking mass. These nests are supposed to form in stagnating blood. Orth and Ponfick failed to find the spirilla in the blood of the cadaver, but in the fresh blood movements may be observed for two to eight hours. Sometimes they are motionless (Engel). Albrecht saw a considerable increase in their numbers in specimens that had stood six hours, and in one of Lachmann's cases a marked increase appeared to occur in the cadaver.

FIG. 37.



Spirillum of Obermeier in human blood. Carbolic fuchsin. Magnified 1000 diameters. (From Slater and Spitta's Atlas.)

Bodies of Uncertain Character Described in the Blood of Recurrent Fever. There has always existed a belief that forms of the parasite other than the spirillum must occur in the blood, and various bodies believed to represent developmental stages of the parasite have been described.

Sarnow and v. Jaksch found, during the afebrile interval, spore-like bodies resembling cocci which became very numerous before the chill. v. Jaksch claimed to have seen these bodies develop into typical spirilla. Larger protoplasmic masses of varying structure were described by Obermeier, Ponfick, Blisener, Litten, Albrecht, and others. Heidenreich described them minutely, as compact, or vacuolated, variously subdivided, of different sizes, containing granules, fat droplets, or red blood cells. Saccharoff also described such masses, finding them most abundant in the afebrile period, some being twenty times the diameter of a red cell. He claimed to have observed the formation of flagella and their separation from the mass. Some of these flagella entered red cells and developed into similar, large, protoplasmic masses, after the manner of the malarial crescent. From the nucleus of the parent mass other slender flagella were protruded, which, on becoming free, circulated in the plasma as the spirilla. Heidenreich regarded these observations of Saccharoff as of great importance in the biological study of the parasite, but they do not appear to have been followed up.

Method of Demonstration. In dry specimens of blood the spirilla stain well with aniline dyes, and Karlinski succeeded in demonstrating flagella with some individuals. Concentrated solution of methylene blue stains all spirilla in two to five minutes.

Examination in the fresh condition yields interesting information regarding the movements of the spirilla, and should be employed to demonstrate their motility, but not for the determination of their presence in doubtful cases.

Occurrence in the Blood. Heidenreich found a few spirilla in the blood twenty-four hours *before the chill*, but most observers have failed to find any at this time.

With the rise in temperature the parasites are usually to be found in small numbers, one parasite in ten to twenty fields of the microscope (Moczutkowsky). They are always to be found on the second day (Blisener), and thereafter rapidly increase, often to enormous numbers (*e. g.*, twenty to thirty in one field). *Yet Engel claimed that even during high fever the parasites cannot always be found in the blood.* During defervescence the numbers steadily diminish, and in the afebrile periods may entirely disappear, but Naunyn, Birch-Hirschfeld, Litten, and Unterberger found an occasional parasite at varying intervals during the *afebrile period.*

With each succeeding paroxysm the spirilla are apt to appear in increasing numbers, but there is no strict parallel between their abundance in the blood and the height of the temperature, while it has been shown that their numbers vary both from day to day, and in different parts of the circulation.

Artificial Cultivation. Heidenreich kept the spirilla alive for one hundred and eighty days in defibrinated blood. The single cultures were preserved longest at a temperature of 18° to 21° C., but at febrile temperature they soon became motionless. They were very sensitive to oxygen, CO_2, saliva, ascitic fluid, urine, and to moderate variations in the quantity of salts in the fluid. Heidenreich and Kannenberg believe this sensitiveness to slight changes in the temperature and osmotic tension of the surrounding medium explains the short life of the parasite in the human body, but Moczutkowsky found them to resist a temperature of 48° C.

Multiplication in artificial media has been successful only in the hands of Koch, but the method was not fully described. Inoculation in monkeys was first successfully performed by Carter and Koch, the animals exhibiting the symptoms of the disease and their blood containing numerous spirilla. Obermeier and Engel had negative results from the injection of blood into dogs, rabbits, and guinea-pigs. Moczutkowsky performed several successful inoculations in men. Very interesting results were obtained by Albrecht, who kept in a moist chamber specimens of blood drawn when the spirilla had temporarily disappeared. After five to six days, in some specimens, the spirochetæ appeared in large numbers. Albrecht describes what he believes to be the intermediate stage of development of the parasite.

Destruction of the spirilla in the blood is accomplished, according to Wernich, by means of the products elaborated by the parasites themselves; according to Heidenreich, through the great susceptibility of the germ to changes in temperature and chemical composition of the blood; according to Moczutkowsky, through concentration of the blood, the density of which, however, diminishes during the paroxysm (Trautgott), and according to Metchnikoff and Soudakiewitch, by phagocytosis.

The paralyzant effects upon the spirilla of the serum of patients who have recovered from the disease indicate that specific bactericidal principles are developed in the blood (Mamourofsky, Gabritschewsky). Yet the studies of Soudakiewitch on phagocytosis in relapsing fever are among the most important on which the doctrine of phagocytosis is founded, and have placed beyond doubt its importance in the destruction of the spirillum of Obermeier.

Changes in Red Cells. Boeckman reported that the red cells sink during and for one to two days after the attack, increasing somewhat in the afebrile periods. Halla counted 4,600,000 red cells on the nineteenth day of the illness, and found 50 per cent. of Hb in a severe case.

Leucocytes. Laptschinsky, Heidenreich, and Boeckman all noted considerable leucocytosis, most marked just after the crisis. Laptschinsky mentioned the presence of a very large number of coarsely granular leucocytes.

Bilious Typhoid Fever (Griesinger). In 1854 Griesinger described under the term *typhus icteroides* a disease which occurred in epidemic form in Cairo, and sporadic cases of which still persist in Smyrna. Moczutkowsky, Karlinsky, Heidenreich, and others fully demonstrated the nature of this malady by finding in every case large numbers of the spirillum of Obermeier.

Serum Diagnosis. Gabritschewsky found that when the blood of a patient who had just recovered from relapsing fever was added to a specimen of blood containing spirilla and kept in the thermostat, the parasites became motionless within one-half to one hour.

Loewenthal applied this fact to the diagnosis of relapsing fever during the apyretic interval when parasites are absent from the blood. The specific reaction was most marked immediately after the paroxysm, diminished steadily, and sometimes became inappreciable, just before the next chill. In cases which had successfully overcome the infection the reaction persisted longer, and Loewenthal claimed that if it persisted as late as the seventh day in sufficient intensity to immobilize the spirilla in one hour no further relapses ever occurred, otherwise relapses invariably followed. There are several uncertainties connected with the work of Loewenthal, and his claims require confirmation.

Sawtschenkow and Melkich find that the bactericidal action of the blood in relapsing fever does not vary with the leucocytes, but increases after the acme of leucocytosis. Heating to 64° C. for one-half hour destroys the immunizing properties of the serum, but does not enfeeble its agglutinating properties.

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CHAPTER XXV.

MISCELLANEOUS PARASITIC DISEASES.

TRICHINA SPIRALIS.

THE relation of trichinosis to the blood is found principally in the extreme degree of eosinophilia which marks the condition.

The **red cells**, in trichinosis, do not suffer greatly. After recovery from the disease, when the disturbing effects of fever are eliminated, a moderate grade of chlorotic anemia is usually to be noted. Thayer reports 4,300,000 red cells and 68 per cent. of Hb in a recent case. In the majority of active cases the red cells are slightly or not at all diminished, while the effects of cyanosis in concentrating the blood have several times been encountered.

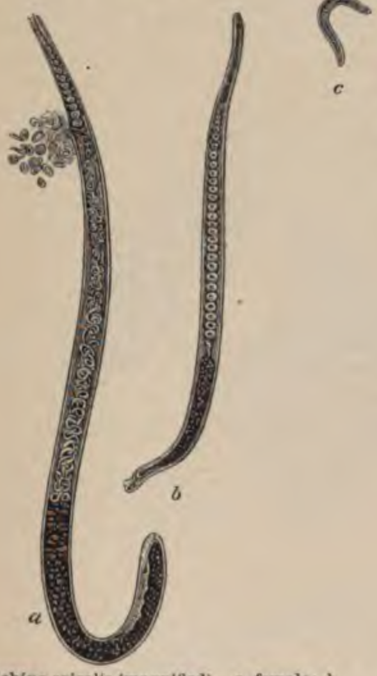
Leucocytes. All cases have shown leucocytosis, ranging from a slight increase to 35,700 cells, recorded by Brown. This leucocytosis is usually in proportion to the severity of the disease (Blumer and Neuman), and persists in moderate degree for weeks or months. It may be more or less intermittent.

The leucocytosis is a natural accompaniment of the exudative myositis caused by the parasite. Brown observed a considerable deposit of oxyphile cells in the neighborhood of some of the cysts and concluded that the transformation of neutrophile cells occurs in the muscles and blood stream, a view which cannot at present be accepted. In Lambert and Brooks' case the writer could find no evidence of the transformation of neutrophile into eosinophile granules, although some of the eosinophile granules were unusually small. In the muscles the writer found a very scanty deposit of eosinophile cells, the majority of new cells being polynuclear or mononuclear leucocytes or proliferated muscle and endothelial cells.

Eosinophilia. Although the presence of marked eosinophilia in various forms of intestinal parasitism was fully demonstrated by Bucklers in 1894, its occurrence in trichinosis was not reported until Thayer and Brown, in 1897, encountered a well-marked case. Since then numerous confirmatory reports have been contributed by Cabot, Gwyn, Atkinson, Stump, Blumer, Neuman, Lambert and Brooks, and others.

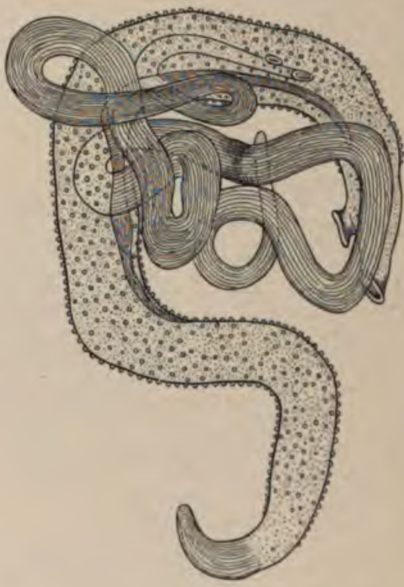
From these observations it appears that trichinosis is invariably accompanied by marked eosinophilia. Yet while the leucocytosis is usually in proportion to the severity of the disease, the eosinophilia bears no such constant relation. One of Blumer's mild cases showed 50 per cent. of eosinophiles, and a recovering case, reported by Brooks, gave 83 per cent. In Kerr's three cases eosinophilia was persistently high, once reaching 86 per cent. of 20,000 cells. Proportions of 40 to 60 per cent. are usually found, but at some periods they may fall to 7 to 10 per cent. It was reserved for Da Costa to record a case of extreme infection without eosinophilia on repeated examinations. In this case the muscle tissue was found to be swarming with trichinæ and rich in eosinophile cells, while the blood gave 12,000 leucocytes and 0.5 per cent. of eosinophiles. As a rule, when the neutrophile cells are abundant the eosinophile are scanty, and *vice versa*. Cabot reports as high as 6 per cent. of myelocytes in this disease. The eosinophilia appears to be very persistent, 15 per cent. of these cells remaining after four and one-half months in Stump's case, and 34.7 per cent. after five months in one of Brown's cases.

FIG. 38.



Trichina spiralis (magnified). *a*, female; *b*, male; *c*, embryo. (From Sahli.)

FIG. 39.



Male bilharzia, with female partly enclosed in gynecophoric canal. (Lortet and Vialleton.

The constancy and extent of eosinophilia in trichinosis render the examination of the blood of great value in the diagnosis of this infection. It seems possible that mild cases have been overlooked in the past which might have been detected by this means.

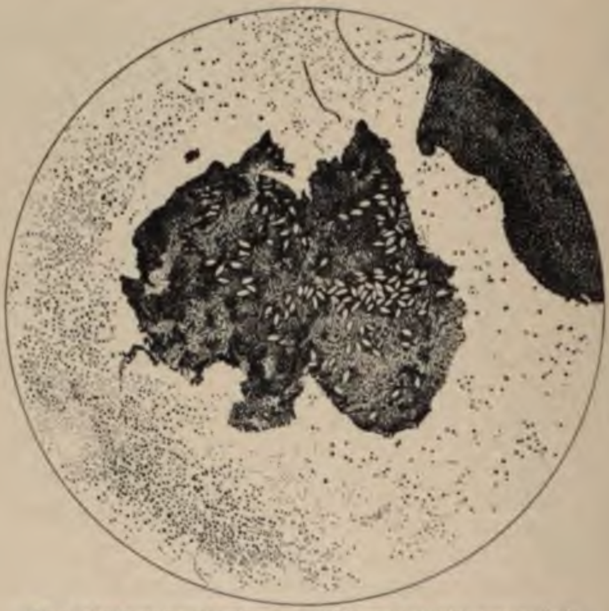
Yet while the presence of marked eosinophilia should always suggest the possibility of trichinosis, there is no great pathognomonic value attached to the symptom, since equal grades of eosinophilia have long been shown to accompany a great many other conditions. The least that must be required in the diagnosis is an unequivocal clinical course, in which case the examination of the blood is secondary corroborative evidence, or the demonstration of eosinophilia in the blood, and trichinæ or exudative inflammation in the muscles (Stump, Brooks).

In the New York Hospital cases have recently been encountered with and without prostration and fever, but with transient edema of the face and moderate eosinophilia. In the mildest of these cases a minute portion of the biceps muscle yielded live trichinæ. This edema, which appears to be a very important diagnostic symptom, was very transient, lasting only a few hours at the beginning of symptoms.

DISTOMA (BILHARZIA) HEMATOBIUM.

This is a nematode worm, very abundant in Egypt and South Africa, and occasionally found in the United States. The male is whitish, 7 mm. to 16 mm. long; the female, often found in the gynecophoric canal of the male, is darker and finer and reaches 20 mm. in length. These worms inhabit the smaller veins of the portal system, where as many as 300 individuals have been found.

FIG. 40.



Blood clot in urine, showing ova of bilharzia (slightly magnified). (After Lortet and Vialleton.)

The **ova**, in the encysted stage, have the shape of a melon seed, $\frac{1}{100}$ to $\frac{1}{200}$ inch in length, with a transparent shell in which the ciliated embryo may be seen. These ova are deposited in the capillary plexuses, where they cause stasis and hemorrhages. They are found in the wall of the bladder, rectum, kidney, ureter, and in the lungs, but never in the spleen, pancreas, or stomach (Lortet, Vialleton). Kartulis found them widely distributed, occurring even in tumors of the bladder, which frequently arise in these subjects. He could never find them in the heart's blood. The ova are usually detected, clinically, in the blood clots which pass with the urine, where their appearance is somewhat characteristic.

The life-history and mode of infection have only partly been traced.

Severe grades of anemia sometimes follow the repeated attacks of cystitis and hematuria.

ASCARIS LUMBRICOIDES (OXYURIS) (TENIA).

Demme reports a case of fatal anemia in a child with 1,650,000 red cells, and large masses of ascarides in the intestines. Bucklers found 41 per cent. of Hb in one case, and in a series of cases counted various proportions of eosinophile cells, maximum 12.25 per cent. In a case of oxyuris he found 16 per cent. of eosinophiles, while in mixed infections of oxyuris and ascaris the proportions were somewhat higher, maximum 19.31 per cent. In cases of tenia, eosinophilia was moderate or absent, the maximum being 10.25 per cent.

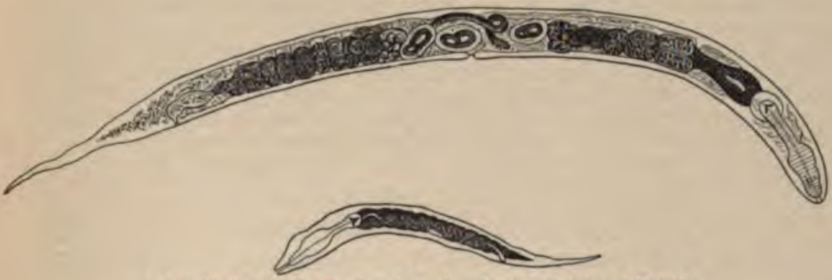
Solley reports two interesting cases of ascarides, in one of which 33 per cent. of 7000 white cells were eosinophile. In the other the patient suffered from pernicious anemia with very large numbers (300 to 35,000) of nucleated red cells, and a distinct normoblastic crisis was observed.

Runeberg has observed one case of pernicious anemia in a patient infected by the *oxyuris*.

ANGUILLULA STERCORALIS (INTESTINALIS).

Anguillula stercoralis is a nematode worm which is often associated with ankylostoma. It measures 2.2 mm. in length, while the embryos are but 0.2 to 0.3 mm. long. The ova develop very rapidly in the intestinal tract, and are passed in the stool only after catharsis. They were believed by Normand, their discoverer, and by Davaine and Perroncito to be the cause of a severe form of diarrhea prevalent in Cochin China, and often accompanied by severe anemia. Manson,

FIG. 41.



Anguillula intestinalis, female and embryo. (Golgi and Monti.)

however, claims that they are innocuous, although Davaine found them present in great numbers in cases of anemia.

Lately Teissier reported the discovery of numerous embryos of *anguillula* in the blood of a case of intermittent fever. With the expulsion of the worms from the intestine the fever declined and the embryos disappeared from the blood. Bucklers reported 13.5 per cent. of eosinophile cells in the blood of one case.

ANKYLOSTOMA DUODENALE.

This parasite is of nearly universal distribution in tropical latitudes, and, although located in the small intestine, secures its nourishment from the blood of the host. It much resembles the ordinary pin worm (oxyuris), being cylindrical in form, 6 mm. to 11 mm. by 0.4 mm. to 0.5 mm. in dimensions (the females are somewhat shorter and thicker), and being white when alive, gray when dead, and reddish brown when full of blood. The head is provided with four hooks, by which the parasite attaches itself firmly to the mucous membrane. The *ova* as found in the feces are similar in general appearance to those of *bothriocephalus*, but the yolk globules are larger.

The *extracorporeal development* passes through several phases, infection occurring by the transfer of earth containing the encysted embryos to the stomach of the host. Leichtenstern has shown that *ankylostoma does not require a second host for the completion of its cycle, but may pass from man to man after a short period of extracorporeal development.* Ova appear in the stools within four to five weeks after the ingestion of the embryos.

Although discovered in 1838 by Dubini, its pathological importance was first established by Griesinger, who, in 1854, showed it to be the specific agent in the so-called "Egyptian chlorosis." Later it was identified as the cause of many forms of tropical anemia, especially by Wucherer in Brazil, and Perroncito in Italy. Through the studies of Grassi, Cinisella, and others, ankylostomiasis became known in Italy as the specific cause of the anemia of mountaineers, tunnel workers, etc., while the discovery by Sonderegger, and others, of the same parasite in the St. Gothard epidemic, called general attention to the subject. In Germany the anemia which had long since been described by Heisse among brickmakers was placed in the same class from the discovery of the ankylostoma by Menche, in an epidemic at Cologne. From these sources the course of infection was traced to nearly all parts of middle Europe. Isolated cases are still occasionally described from these regions, while the disease remains epidemic in many tropical regions. The disease is indigenous in the United States.

Mode of Infection. The chief source of infection is by the transfer of the embryos to the mouth by dirty hands. The rapid spread of the disease and its contagiousness are explained by the rapid development of the ovum, which, on leaving the host, reaches the encysted form, capable of further infection, within one or two weeks (Manson). A second mode of infection, pointed out by v. Schopf and demonstrated experimentally, consists in the inhalation of the encysted larvæ *in dust*. In the alkaline fluids of the duodenum the chitinous hull of the embryo is dissolved, when the young worm promptly attaches itself to the mucosa. Only the plasma appears to be utilized in the nourishment of the parasite, as undissolved red cells are extruded from the anus (Leichtenstern[2]). The number of worms found in cadavers varies greatly, but often several hundred are to be counted, the males being one-third as numerous as the females. Leichtenstern[1] counted as many as 3000 in a fatal case.

Pathogenic Action. The essential action of the parasite in the causation of anemia has been repeatedly shown by the prompt recovery which has followed its expulsion. Yet the anemia has progressed in some instances after the expulsion of the worm, and a cure cannot be assured in the very advanced stages of the disease. The frequent losses of blood are undoubtedly a chief cause of anemia, since the parasite, though small, is very prodigal of the blood, and the severest cases usually show most parasites. Yet there have been many exceptions to this rule, and some fatal cases having shown very few parasites, like that of Leichtenstern, in which only twenty-four were found, it has been held that a condition of *specific intestinal intoxication* is established by the growth of the worms. The evidence on which this theory is based is, however, very incomplete. Lussana found that the urine in ankylostomiasis, when injected into rabbits, dissolves red cells much more actively than does normal urine, and Bohland demonstrated that there is a very active destruction of albumins in this form of anemia.

In Sandwith's remarkable experience the red cells averaged 1,300,000, while the Hb even with very low numbers of red cells was much reduced, average 26 per cent. The condition of the blood was, therefore, not strictly in accord with that of progressive pernicious anemia, while the pathological lesions found at autopsy also varied from the typical features of pernicious anemia. In Ashford's report, among important features, are a record of 668,888 red cells, excessive reduction in Hb with extreme anemia, and the rapid progress of the anemia followed by rapid improvement on the expulsion of the worms. The usual degenerative changes in the red cells have been uniformly present in severe cases. Ashford found rather few nucleated red cells as compared with idiopathic anemia, and always a predominance of normoblasts.

Changes in the Blood. The anemia passes more or less rapidly through milder stages, and in severe cases reaches the pernicious grade.

In the majority of instances the condition observed is one of chlorotic anemia, with moderate loss of red cells and very marked loss of Hb. In cases with over 4,000,000 red cells Zappert found 50, 40, and 30 per cent. of Hb. In many cases, however, the changes in the blood are more severe, and the presence of megalocytes, microcytes, megaloblasts, and increased Hb-index renders the condition identical with cryptogenic pernicious anemia.

In the progress of the anemia Leichtenstern distinguished two stages, which can be most clearly traced when the infection is very active. There is an *initial stage of acute anemia* when the parasites are actively changing their location in the intestine, which is marked clinically by colicky pains and bloody diarrhea. Later comes the *stage of chronic anemia*, maintained by the steady consumption of blood by the parasites as well as by some intoxication.

Leucocytosis of moderate grade is frequently observed, especially during the early stages. That it is absent in the majority of uncomplicated cases is apparent in the study by Ashford, who is inclined to refer any leucocytosis in the disease to complications. Bucklers found 20,000 white cells in an early case, and many others have reported intermediate grades of leucocytosis. In the severe grades of anemia the leucocytes are usually diminished, Zappert[1] finding only 1800 in one instance, and Ashford 1500 in his severest case. The eosinophile cells are nearly always in excess and sometimes reach an extreme proportion This fact was first noted by Müller and Rieder, and Zappert,[1] but a very high proportion (53 per cent.) was first recorded by Bucklers, while Leichtenstern[1] saw cases with 62 and 72 per cent.

BOTHRIOCEPHALUS LATUS.

Description of Parasite. This tapeworm measures from six to ten metres, occasionally twelve to sixteen metres in length, and is distinguished from all other intestinal parasites by the combination of a very broad segment and a pigmented uterus which occupies a central position in the segment. The head has neither rostellum nor hooklets. The eggs are oval, 0.05 mm. by 0.035 mm., with a rather thin, double-contoured shell, at one end of which is a cap, while the undeveloped embryo is found to consist of a number of large, yellowish globules. Infection occurs by eating uncooked fish, which is the intermediate host. This practice is confined largely to the French, Swiss, and Italian lakes, the shores of the Baltic and North seas, and Japan, but owing to the long life of the parasite infected subjects have been encountered in many other countries. The writer obtained four adult specimens from a healthy Swede in New York City who had not been out of the State for eleven years.

History and Pathogenesis. The first important inroad made upon the group of idiopathic pernicious anemia was accomplished when Hoffman, in 1885, followed by Botkin, Reyher, and Runeberg, showed that the presence in the intestine of many of these worms often leads to a severe or fatal anemia which may be made to disappear by the expulsion of the worms. While Reyher and Runeberg, in 1886, and F. Müller, in 1889, claimed that the morphology of the blood in these severe cases is identical with that of primary pernicious anemia, from the presence of megalocytes with increased Hb, megaloblasts, etc., the complete identity of the condition existing in fatal cases with that of progressive pernicious anemia was fully established by Schaumann's extensive studies in the same locality. It is of great interest that in some of Schaumann's cases, although the reduction of cells and Hb was not marked, yet Ehrlich's signs of progressive anemia, megalocytes, with increased Hb and megaloblasts, were abundantly present.

While the fact that the worms cause the anemia seems to be proved by the rapid recovery observed in some cases following their expulsion (Wiltschur, Askanazy), no satisfactory explanation of the pathogenic action of the parasites has been obtained. It appears that large numbers of worms are required to produce the severe anemias, as many as 100 having been expelled from a patient of Boetticher, although in other instances the usual small number was found. The duration of the infection appears to exert little influence, as is evident from the cases reviewed by Askanazy. The writer secured four full-grown species from a healthy Swede who had been in America for seventeen years. Schapiro and Dehio, finding some dead parasites in their cases, concluded that only the *decomposing worm* produces the specific toxin which causes the anemia, but the majority of parasites found in severe cases are living. Wiltschur followed this clue further, finding that the majority of parasites were dead and decomposing, or, as he concluded from alterations in the eggs, "sick." Some cases (Schaumann, Neubucher) in which anemia was present while no parasites, but only eggs, were found in the intestines at autopsy, Ehrlich believes can be explained as resulting from the complete absorption of recent decayed parasites. This reasoning it is difficult to follow, nor can great importance be attached to the results of Schaumann and Tallquist, who in the course of two weeks considerably reduced the red cells of a dog by injections of a glycerin extract of the crushed bodies of the worms.

At present, therefore, it seems impossible to reach any satisfactory explanation why this parasite should behave at times as a harmless denizen of the intestinal tract, but at others should destroy its host through a fatal anemia.

Changes in the Blood. In the great majority of Schaumann's cases the changes in the blood were characteristic of pernicious anemia. The red cells at first ranged from 2,150,000 to 395,000, an average of 1,290,000. They commonly exhibited marked changes in size; there was unusual excess of megalocytes with increased Hb, and these changes were sometimes present when the reduction in cells and Hb was not marked.

A surprising improvement usually followed the expulsion of the worms, and most of the cases were discharged practically cured and apparently removed from danger of relapse. In some of Schaumann's cases, however, as in others reported by previous observers, the anemia progressed in spite of the expulsion of the worms.

Nucleated red cells were present in all cases, and usually, but not always, the megaloblasts outnumbered the normoblasts. In Askanazy's cases normoblasts were found in small numbers among many megaloblasts, but the former rapidly replaced the latter as the anemia improved. Willson found many normoblasts in a case showing 5,120,000 red cells and 60 per cent. Hb. Polychromasia and granular degeneration were pronounced at the height of the anemia, but diminished rapidly as the blood improved. The Hb was usually below 30 per cent., average at first 25 per cent., and the Hb-index was almost always normal or increased.

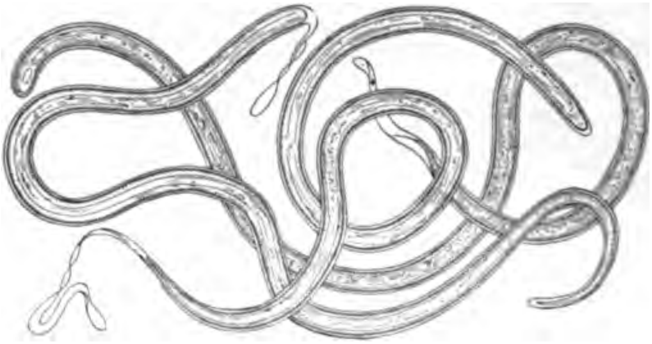
The leucocytes were very seldom increased, and eosinophile cells, if present, did not attract the attention of Askanazy or any of the earlier observers, but were reported as increased in one of Schaumann's cases.

FILARIASIS.

Several species of nematode worms inhabit the internal vessels and discharge their embryos into the circulation, giving rise to the condition known as filariasis. The embryos of the several species exhibit characters which should render possible their accurate identification.

According to Manson these features are principally (1) periodicity in the presence of the embryos in the peripheral blood. *Filaria diurna* appears during the day and disappears at night *Filaria nocturna* appears at night only. *Filaria perstans* and *Demarquaii* are constantly present, both by day and by night. (2) The presence or absence of a sheath which characterizes all species except *Filaria perstans*. (3) The character of the head and tail ends; the presence or absence of an armature; the blunt or pointed shape; the length of the tapering end. (4) Length, breadth, and general appearance of body. (5) Character of movements. *Filaria noctura* and *diurna* are lashing, but stationary. *Filaria perstans* lashing, but locomotor. The associated pathological condition, the nativity of the patient, and the character and location of the parent worm, if found, are also important.

FIG. 42.



Filaria nocturna, with sheath. (Lewis.)

Filaria Nocturna. **Developmental Cycle.** The embryo is taken up from the blood of the host by the mosquito, in whose stomach it becomes rid of its sheath, and at the end of six to seven days develops in the viscera of the insect to a length of 1.58 mm., and acquires four lips and an alimentary canal. The further stages between this form and the adult filaria of Bancroft have not been traced. The worm is next found in the lymphatics of the trunk and extremities, where it was first seen by Bancroft (*F. Bancrofti*). Here it measures 70 mm.

(males) to 94 mm. (females) in length, and looks like an animated white thread. Its movements are active and wriggling, the female tending to coil. The young filaria are discharged into the lymph, and, traversing the lobes, reach the blood stream. The reason of their exclusive appearance during the night is not known.

Usually the presence of this worm and its embryos leads to no appreciable disturbance in the body of the host, and numerous cases have been discovered entirely by accident. In some cases chronic inflammation of the lymphatics results in a variety of pathological conditions, principally chyluria, lymphangitis, lymph scrotum, etc., and probably also to *elephantiasis Arabum.*

The embryo of *Filaria nocturna* is a slender, snake-like worm, 3 mm. by 0.0075 mm. in dimensions, with sharp-pointed tail tapering for about one-fifth the length of the body, and a blunt head. The head is composed of a six-lipped prepuce covering an extensive proboscis on which is a protrusible spine. The entire worm is enclosed in a delicate, hyaline, transparent sheath, which protrudes beyond head or tail, and within which the animal moves. This sheath exhibits fine cross-striations. The movements are actively lashing, writhing, coiling, and uncoiling, *but with little or no tendency toward locomotion.* The movements continue for several hours, or until the blood suffers drying.

Occurrence in the Blood. The embryos begin to make their appearance in the blood from five to seven o'clock in the evening. The numbers increase for several hours during rest in bed, and after midnight they usually become less abundant, disappearing at seven or eight in the morning, but an occasional specimen may sometimes be found at any time in the day. This remarkable periodicity depends on the habits of the host, since it becomes transposed when the subjects sleep in the daytime, and it is considerably disturbed by irregular habits and by fever. It is also adapted to the nocturnal habits of the mosquito. The *numbers of filaria to be found in the blood* depend upon the time of the examination and upon the number of parent worms, but for the same patients they are usually quite uniform. From ten to fifty may be found under one cover-glass specimen, but they are often much less abundant. Several hours' search through thick blood smears may be required for the demonstration of a single filaria embryo, in cases in which they are present, though very scarce. It is sometimes to be observed that the results of the blood examination are negative as long as the patient is going about, although a moderate number of embryos are found after the patient has been in bed a few days.

Changes in the red cells referable to the presence of filaria embryos are not usually demonstrable. Even when present in enormous numbers and for many years the parasites exert no deleterious action on the blood, and anemia, when present, must be referable to secondary causes.

Calvert examined the blood at hourly intervals for twenty-four hours in two cases of filariasis, finding leucocytosis, reaching 26,666 and 14,000. The eosinophile cells varied from 8 to 22 per cent. in one case and from 3 to 20.5 per cent. in the other. The leucocytosis and excess of eosinophile cells were most marked during the daytime, when the embryos in the peripheral circulation were scanty. He thinks that eosinophilia diminishes as the infection becomes chronic.

Method of Demonstration. On account of the large size and active movements of the filaria, examination of the fresh blood may be recommended in the search for the parasite when its presence is suspected. Fresh specimens prepared in rather thick layers should be looked over with a moderately low-power lens. Permanent specimens may be secured by smearing the blood on glass slides, fixing by heat or alcohol, and staining by methylene blue. Canada balsam causes fading after a year or more, and Manson uses glycerin jelly as a mounting medium.

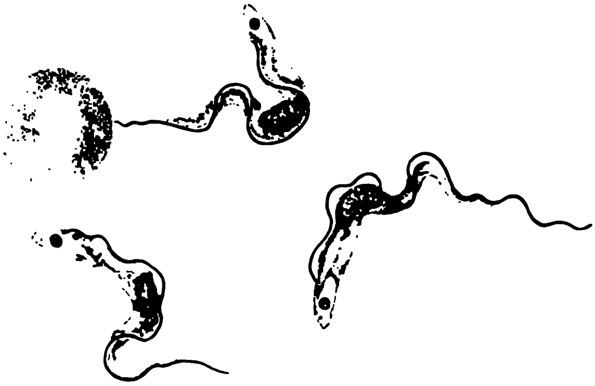
Filaria Diurna. In four cases of filariasis occurring in negroes on the coast of Africa, Manson observed filaria resembling *Filaria nocturna* in the blood only between 8 A.M. and 9 P.M. He suggests that the *Filaria loa* is the parent form, since one of his cases had previously suffered from this parasite, and that the "mangrove fly" is the intermediate host.

Filaria Perstans. In some portions of the west coast of Africa Manson found that two-thirds of the natives are infected with a special form of filaria. The embryos are thinner and longer than *Filaria nocturna*, 23 mm. by 0.0045 mm.; they lack a sheath, execute movements, often very rapid, of *locomotion*, occur in much smaller numbers in the blood, and are found both by day and by night.

TRYPANOSOMIASIS.

Although infection of the blood by various forms of trypanosomes has long been recognized in several tropical diseases of domestic animals as surra, nagana, "mal de caderas," etc., the occurrence of pure infections by a form of this parasite in the blood of man has

FIG. 43.



Trypanosoma gambiense in human blood. (After Dutton.)

only been satisfactorily demonstrated within the past year, 1902–03, when no less than eleven authentic cases have been reported by Dutton, Forde, and Manson.[2] Nepveu, however, reported in 1898 the discovery in the blood of six malarial patients of bodies having all the characters of trypanosomes.

In man the disease follows a chronic course with intermittent fever, marked *erythema multiforme* of trunk and limbs, edema of eyelids and ankles, emaciation, and enlargement of the spleen.

Morphology and Occurrence of the Parasite. In stained preparations the parasite and its flagellum measured in Dutton's case 18μ to 25μ in length and 2μ to 2.8μ in width. The posterior end of the wormlike body is blunt and conical, while the anterior end is pointed. An oval nucleus consisting of chromatin grains occupies a central position, while in the posterior end lies a centrosome or micronucleus giving origin to the flagellum which curves forward along the free border of the undulating membrane of the parasite and projects some distance beyond the anterior end of the body. By Romanowsky's method these structures stain like those of the malarial organism. The undulating membrane is an achromatic structure whose free border is continuous with the flagellum while the attached border begins at the centrosome and runs forward to the pointed end of the body. The web is transparent. The parasite exhibits slow spiral movements of progression. They are never found in the red cells, but may be englobed and destroyed by the leucocytes. Multiplication has been observed in experimental infections in rats, and occurs by transverse and longitudinal division and by segmentation (Rabinowitsch and Kempner, Francis). Infection in man is probably carried by mosquitoes (Dutton) and in rats by fleas, lice, and by the food.

The number of parasites seen in the blood is scanty, not more than three to eight being found under a three-quarter inch cover-glass, while during apyrexia they were not found at all. Repeated examinations may be required for their detection.

The Hb and red cells are moderately reduced, the leucocytes slightly increased, with relative lymphocytosis. In Dutton's case the following results are reported: Hemoglobin, 76 per cent.; red cells, 3,850,000; leucocytes, 12,000, of which 60 per cent. were mononuclear, 40 per cent. polynuclear.

SPOTTED FEVER OF MONTANA.

According to the observations of Wilson and Chowning this peculiar local disease is referable to infection of the red blood cells by an hematozoon resembling the parasite of Texas cattle fever.

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APPENDIX.

DURING the passage of this volume through the press contributions appearing in certain lines call for the additional paragraphs appended.

Dare's Alkalimeter.

Dare (*Philadelphia Medical Journal*, vol. xi. p. 137) has devised a commendable method and apparatus for the clinical estimation of the alkalinity of blood. Its principal features are the avoidance of a foreign indicator, the point of neutralization by tartaric acid being determined by the disappearance of the spectroscopic absorption bands of oxyhemoglobin, the use of an automatic 20 c.mm. blood pipette, and the absence of undue exposure to air of the manipulated blood.

Further Observations on Polychromasia and Granular Degeneration of Red Cells.

The observations on polychromasia and granular degeneration of red cells seem to indicate a certain relation of these processes to each other and to developmental anomalies in the erythrocyte. While present knowledge hardly warrants positive conclusions, one will probably not be greatly in error who conceives of them all as the expressions of one series of changes. It would appear that the imperfectly developed red cell may be deficient in Hb, or may contain imperfectly oxidized Hb (Sherrington), which give it the character of the polychromasia of Maragliano; or that a portion of the cell may be entirely free from Hb (polychromasia of Gabritschewsky); or the imperfectly developed red cell, while deficient in Hb, may show a clumping of its nuclear constituent in the form of fine basic granules (granular degeneration), while the irregular diffusion of nuclear elements in the megaloblast occasionally yields large and small basophile granules in the cytoplasm interpreted by some as an evidence of karyorrhexis. That all of these changes frequently occur in imperfectly developed cells is beyond question, while the belief that red cells once perfectly formed may suffer such alterations seems much less certain. The writer is inclined to look upon all these changes not as occurring in the circulation, but as the result of disturbances of the function of red-cell formation in the marrow.

The results of Schmidt's experiments (*Exper. Beitr. z. Path. d. Blutes*, Jena, 1902), showing that after the intravenous injection of lead salts granular degeneration does not occur in the red cells of the ligated ear, strongly support this view and indicate that these granules are nuclear particles representing an anomaly of development.

Leukanemia.

This is a term suggested by v. Leube to indicate certain cases of rapidly fatal anemia in which the blood shows pronounced characters of pernicious anemia, the moderate leucocytosis of acute lymphemia or myelemia, while the viscera exhibit distinct leukemic lesions. In this group fall the majority of cases of acute leukemia, cases of pernicious anemia described as terminating in leukemia, cases of lymphatic leukemia without lesions in the lymph nodes, and possibly some cases of v. Jaksch's anemia. The typical case is characterized by peracute or subacute course, fever, gingivitis, and hemorrhages. The blood first shows the changes of pernicious anemia, with slight or no leucocytosis. Later there is moderate or pronounced increase of large lymphocytes or myelocytes. Eosinophile cells are absent or scanty. There are moderate hyperplasia of lymph nodes and spleen, extreme diffusion of red marrow, and leukemic deposits in the liver. Siderosis of the liver and Charcot-Leyden crystals are absent. It is believed that the condition results from the action of a toxic or infectious agent upon the progenitors of the red and white cells. The possibility that the progenitors of the red and white cells constitute a single cell type in the adult marrow, and the striking clinical characteristics of these cases, are at least a partial warrant for the general introduction of this term. Leukanemia is, then, a term referring to a disease which may be regarded provisionally as constituting a point of union of leukemia and pernicious anemia.

v. Leube. Deut. Klinik, 1902, Lief. 42. Münch. med. Woch., 1900, p. 1121.
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Serumtherapy in Anemia.

Bielonovsky reports very striking improvement of the blood in nine cases of primary pernicious and severe secondary anemia by injections of 5 c.c. to 10 c.c. of hemolytic serum prepared in the rabbit.

Bielonovsky. Thèse de Doctorat, ref. in Bull. Instit. Pasteur, 1903, p. 126.

Bacteriological Study of the Blood in Scarlet Fever.

Hektoen reports the isolation of streptococci from the circulating blood of 12 of 100 cases of scarlet fever, most frequently in severest cases. In two cases of the series pure cultures of staphylococcus aureus were obtained, and in one case bacillus typhosus. Weaver and Ruediger find that neither normal nor scarlatinal blood serum has any bacteriolytic action on various strains of streptococci.

Hektoen. Journ. Amer. Med. Assoc., March 14, 1903.
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Bacteriology of the Blood in Pneumonia.

The following additional studies of the blood from the basilic vein in pneumonia favor the conclusion that this disease is in the great majority of cases a form of bacteremia.

Casati demonstrated the pneumococcus in the blood of 25 consecutive cases by inoculation in mice. Positive cultures are reported in all of 8 cases by Berghini, in 55 of 57 cases by Baduel, and in 74 of 83 cases by Rosenow. The last observer finds it possible to identify the pneumococcus in smears of the blood in many cases.

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Sleeping-sickness.

Castellaini and Bruce report the discovery of trypanosomes in the aspirated cerebrospinal fluid of all of 38 cases, and in the blood of 12 of 13 cases examined. They believe this infection to be the cause of the disease, while the streptococcus is a frequent secondary infecting agent. This trypanosome differs slightly from that described by Dutton.

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INDEX.

- ABSCESS, 326
  - of liver, 392
- Absorption, effects on blood, 402
- Acetonemia, 83
- Acidemia in carcinoma, 91, 426
- Acidity of blood, 92
- Actinomycosis, 335
- Addison's disease, 375
- Agglutinins, 145
- Albumins of serum, 75
- Albumose, occurrence in blood, 77
- Alexines, 128, 140, 144, 158
- Alkalescence of blood, Engel's alkalimeter, 60
  - estimation of, 59
    - Dare's method, 483
    - Limbeck's method, 61
    - Lowy's method, 59
    - Schultz-Schultzenstein's method, 60
    - Wright's method, 61
  - in carcinoma, 91, 426
  - in cholera, 404
  - in diabetes, 374
  - in fever, 91, 92, 279
  - general factors in, 90
  - in gout, 377
  - in hemoglobinemia, 369
  - in leukemia, 247
  - in uremia, 418
- Amboceptor, 144, 158
- Anadenia, 209
- Analyses of blood, interpretation of, 17
- *Ankylostoma duodenale*, 474
  - anemia from, 206, 475
- Anemia, from *Bothriocephalus latus*, 206
  - changes in blood, 220, 476
  - infantum pseudoleukemica, 268
    - pathological anatomy, 271
  - lymphatic, 171, 344
  - progressive pernicious, 202
    - changes in blood in, 216
      - chemistry, 216
      - Hb, 217
      - leucocytes, 221
      - red cells, 218
    - etiology, general, 204
      - special, 206
    - gastro-intestinal diseases in, 208
    - hemorrhages as a cause of, 207, 355, 394, 398, 475
- Anemia, progressive pernicious, historical, 202
    - malaria as a cause of, 208
    - marrow lesions in, 212
    - nervous system in, 211
    - pathogenesis of, 215
    - after pregnancy, 207
    - résumé of chief facts, 222
    - scope of term, 205
    - typhoid fever as a cause of, 208
  - secondary, eosinophiles in, 162
    - origin of, 117
  - of v. Jaksch, 268
    - significance of, 270, 484
  - splenic, 264
- Anesthesia, 110
- *Anguillula stercoralis*, 473
- Anhydremia, 17
- Anisocytosis, 97
- Anthrax, 335
- Antialbumid, 73
- Antifebrin poisoning, 366
- Antipyrin poisoning, 366
- Antitoxin in diphtheria, effects on blood, 292
- Antitoxins, etc., 145
- Appendicitis, 325
- Arsenic, effect on red cells, 113, 363
- Arseniuretted hydrogen, poisoning by, 363
- *Ascaris*, 206, 473
- Ascites, 394
- Ash of blood, inorganic principles, 78
- Asphyxia, 408
- Asthma, 409
  - eosinophilia in, 162, 409

- BACTERIA, action of, on blood, 279
  - in blood of pneumonia, 289, 485
  - in cholera, 404
  - in chorea, 386
  - in endocarditis, 413
  - in influenza, 333
  - in leprosy, 350
  - in leukemia, 232
  - method of examining blood for, 67
  - in purpura hemorrhagica, 354
  - in rheumatism, 328
  - in scarlet fever, 300, 484
  - in scurvy, 358
  - in septic processes, 323

Bacteria in tuberculosis, 348
  in typhoid fever, 306
Bactericidal action of blood, 139
Banti's disease, 265
Barlow's disease, 359
Basedow's disease, 387
Basic capacity of blood, 92
Basophilia, perinuclear, Neusser's, 132
Baths, cold, in typhoid fever, 304
Beriberi, 386
Bibliography, carcinoma, sarcoma, 428
  chemistry, 93
  chlorosis, 200
  constitutional diseases, 382
  development of blood cells, 188
  diphtheria, 308
  eosinophilia, 173
  exanthems, 308
  fever, 280
  general physiology, 68
  heart, 418
  hemopoietic system, 405
  hemorrhagic diseases, 369
  infectious diseases, 336
  v. Jaksch's anemia, 272
  kidneys, 418
  leprosy, 350
  leucocytes, leucocytosis, 158
  leukemia, 251
  lungs, 418
  lymphocytosis, 173
  malaria, 464
  morphology and physiology, 120
  nervous and mental diseases, 387
  parasitic diseases, 481
  pernicious anemia, 224
  pneumonia, 308
  pseudoleukemia, 266
  relapsing fever, 469
  splenectomy, 276
  syphilis, 350
  tuberculosis, 350
  typhoid fever, 308
  Widal's test, 319
Bile, detection of, 84
  effects of, on blood, 389
Bilharzia hematobia, 472
Blackwater fever, 367
Blood crises, 195, 220, 222, 321
  dust, 133
  islands, 175, 213,
  plates, chemistry of, 74
    development of, 186
  smears, fixation of, 54
Bones, tuberculosis of, 348
Bothriocephalus latus, 476
    anemia from, 206, 220, 476
Breast, carcinoma of, 425
Bremer's reaction, 64
Bronchitis, 410
Burns, 362

CARBONIC dioxide, occurrence in blood, 90
Carbonic oxide Hb, chemistry of, 72
Carbonic oxide Hb, spectrum of, 30
    poisoning, 366
Carcinoma, 421
  acidemia in, 91, 426
  alkalescence in, 426
  of esophagus, 394
  of breast, 425
  of liver, 391
  special factors in anemia of, 423
  specific gravity in, 426
  of stomach, 399, 209
    digestion leucocytosis in, 401
Centrosomes, 127
Charcot-Leyden crystals in leukemia, 248
Chemistry of blood, 71
  in diabetes, 373
  in gout, 376
  in leukemia, 247
  in myxedema, 381
  in nephritis, 416
  in osteomalacia, 377
  in pernicious anemia, 216
  in pneumonia, 283
  in rachitis, 380
  in septic processes, 322
  in tuberculosis, 348
  in uremia, 417
Chemotaxis, 134
Children, *vide* Infants.
Chlorosis, 191
  chemistry, 196
  Egyptian, 474
  eosinophiles in, 162
  etiology, 191
  Hb, 194
  iron in, 112, 193, 197
  leucocytes, 198
  marrow in, 196
  nervous system in, 192
  nucleated red cells, 195
  pseudo-, 199
  red cells of, 194
  regeneration of blood, 197
  specific gravity, 193
  thyroid, 387
  varieties of, 198
Cholelithiasis, 392
Cholemia, occurrence and detection of, 83
Cholera, 403
  alkalescence in, 404
  bacteria in, 405
  infantum, lymphocytosis of, 397
Chorea, 386
  bacteria in, 386
Chromic acid poisoning, 365
Cirrhosis of liver, 392, 393
Coagulability, determination of, 61
  in hemoglobinemia, 369
Coagulation, in blood of fever, 280
Cold, effect in concentrating blood, 19, 109 304
Colitis, 403
Coma, in cancer, 426
  in diabetes, 373, 374

Coma in malaria, 456
    in uremia, 417
Complement, 139, 144, 158
Concentration of blood, effect of cold on, 19
        of electricity on, 19
        of massage on, 19
Conjugation of malarial parasites, 454
Constitutio lymphatica, 170, 191
Constitutional diseases, 353, 372
Convulsions, 385
Crioscopy, 62
Cytases, 140, 144

DARE'S alkalimeter, 483
    hemoglobinometer, 51
Defibrinated blood, transfusion of, 364
Depletion, effects of, on blood, 402
Dementia, 384
Development of blood cells, 175
Diabetes, 372
    alkalescence in, 374
    Bremer's reaction in blood of, 64
    chemistry of, 373
    glycogen in, 374
    jecorin in, 374
Diarrhea, 403
Diarrheal diseases, polycythemia of, 109, 403, 409
Diastatic ferment, 87
Digestion, effect on red cells, 105
    leucocytosis, 146, 401
Diphtheria, 290
    antitoxin in, effects of, on blood, 292
    degeneration of leucocytes in, 292
    lymphocytosis in, 291
Distoma hematobium, 472
Dry specimens, 53
Duodenal ulcer, 402
Dysentery, 403
Dyspepsia, 397

ECHINOCOCCUS, in liver, 393, 165
Electricity, effect in concentrating blood, 19
Emphysema, 410
Empyema, complicating pneumonia, 284
Endocarditis, 410
    bacteria in, 413
    gonorrheal, 413
    malignant, 412
    polycythemia of, 109, 411
Endometritis with sepsis, 321
Eosinophilia, 161
    in ankylostomiasis, 165, 474
    with ascarides, etc., 473
    in asthma, 162, 409
    in diagnosis, 167
    in leukemia, 162, 243
    marrow changes in, 166
    origin and significance of, 166
    in pneumonia, 286
Eosinophilia, post-febrile, 164
    in rachitis, 380
    relation to lymphocytosis, 172
    in sarcoma, 163, 428
    in scarlet fever, 164, 299
    in trichinosis, 165, 470
Eosinophile cells, chemistry of, 186
    development of, 166, 185
    estimation of, 42
    proportions, 161
    structure, 125
Epilepsy, 384
    lymphocytosis in, 385
Erysipelas, 324, 326
Esophagus, lesions of, 394
Ether, 110
Exanthemata, 294
    eosinophiles in, 164

FAT, demonstration of, 67
    occurrence and estimation of, 83
Ferments, 86, 139
Ferrometer, Jolles', 52
Fever, alkalescence in, 91, 92, 279
    blood in, 164, 277
    hemocytolysis in, 277
Filariasis, 478
Fixation of blood smears, 54
Flagellate bodies, preparation of, 431
Fleischl's hemoglobinometer, 45

GASTRITIS, 396
Gastro-intestinal diseases, 394
        and pernicious anemia, 208
        of infants, 397
Glanders, 335
Glucose, occurrence and estimation of, 81
Glycogen, demonstration of, 66
    in diabetes, 374
    in leukemia, 248
    occurrence, 81, 248, 323, 326, 374
    in pneumonia, 288
Glycolytic ferment, 81, 87, 374
Goldhorn's stain, 435
Granules, Ehrlich's, 126, 128
Gonorrhea, 331
    eosinophiles in, 165
Gout, 376
    alkalescence in, 377
    chemistry of, 376
    eosinophiles in, 165, 376
    uric acid in blood of, 377
Gowers' hemoglobinometer, 43
Graves' disease, 387
Guaiac test, 24
Guaiacol, poisoning by, 364

HB, hemoglobin, 29
    chemistry of, 71
    estimation of, 43
    spectrum of, 29
Heart, congenital disease of, 414

Heart, diseases of, 410
Hemameba immaculata, 444, 449
Hematin, chemistry of, 73
spectrum of, 29
Hematoblasts, 177
Hematocrit, 31
Hematocytometer, 35
Oliver's, 40
Thoma's 35
directions for using, 37
sources of error, 39
Hematoidin, chemistry of, 73
in globulicidal serum, 76
Hemin test, 24
Hemocytolysis, 118, 359, 426
in fever, 277
Hemoglobinemia, 72, 221
alkalescence in, 369
coagulability in, 369
malarial, 367
Hemoglobinometer, 43
Dare's, 51
Fleischl's, 45
Gowers', 43
Haldane's, 44
Miescher's, 47
Oliver's, 48
Tallquist's, 44
Hemoglobinuria, 366
Hemokonia, 133
Hemolymph nodes, 183
Hemolysins, 144
Hemophilia, 356
Hemorrhage, a cause of pernicious anemia, 207, 394, 398, 475
changes in blood from, 114
Hemorrhagic diseases, 353
Hemosiderin, chemistry of, 73
Histon, chemistry of, 74
Hunger, blood in, 396
Hydremia, febrile, 278, 410
Hydrobilirubin, 79
Hydrocyanic acid poisoning, 366
Hyperacidity, gastric, 396
Hypochondriasis, 387
Hypoleucocytosis, experimental, 136
in pneumonia, 286
in septicemia, 322
Hysteria, 387

IMMUNE body, 139, 144
Immunity, 138
Ehrlich's theories of, 141
Infants, atrophy of, 264, 395, 397
characteristics of blood, 270
cholera infantum, 397
digestion leucocytosis, 147
eosinophiles in blood, 161
hemorrhage, effects of, 116
leucocytes, fetal, 184
proportions of, 129, 161, 170
leucocytosis of newborn, 149
leukemia, occurrence of, 230
liver, formation of red cells in, 180, 270, 389
Infants, lymphocytes in, 170, 171
lymphocytosis of infectious diseases, 171, 287, 291
marasmus, 264, 395, 397
marrow, limits of red, 180
megaloblasts, occurrence of, 98
melena neonatorum, 343, 355
normoblasts, occurrence, 98
pernicious anemia in, 206, 207
polychromasia in fetal red cells, 101
pseudoleukemia in, 258
purpura hemorrhagica in, 354
red cells, numbers, 107
size, 97
spleen in anemia, 180
splenomegaly, 264
Influenza, 332
bacteria in, 333
Inorganic principles of blood, estimation of, 80
Interpretation of analyses of blood, 17
Intestinal intoxication in leukemia, 231
Intestine, diseases of, 402
Iron in blood, estimation of, 52
in chlorosis, 112, 193, 197
effect of, on red cells, 111
indications for, 112
occurrence of, 79
Isolysis, 361

JAKSCH, v., anemia, relation to pseudoleukemia, 268
Jaundice, 390
polycythemia in, 391
Jecorin, in diabetes, 374
Jenner's stain, 56
Jolles' ferrometer, 52
Justus' test, 341

KIDNEY, diseases of, 414
Kreatinin, 418

LEAD, effects on blood, 113
Leprosy, 349
bacteria in, 350
serum diagnosis of, 350
Leucocidins, 362
Leucocytes, acute degeneration, 130
chemistry of, 73
chronic degeneration, 131
classification, 123, 126
degeneration of, in diphtheria, 292
in pneumonia, 288
degenerative changes in, 130, 242
development of, 180, 166
of varieties, 184
diminution of neutrophile granules, 131, 242
estimation of, 41
in same preparation with red cells, 42
fatty degeneration, 132
finer structure, 127

Leucocytes, fragmentation of bodies, 131
  hydropic degeneration, 132, 242
  morphology, 123
  neutrophile, development of, 184
    structure, 124
  nuclear changes, 131, 242
  numbers, 128
  in pathological blood, 125
  pigmented, in malaria, 463
  polynuclear, degenerative changes in, 131, 242
  proportions of forms, 129
  "shadows," 131, 288, 291
Leucocytosis, antemortem, 154
    in leukemia, 246
  cachectic, 152
  changes in marrow, 135
  classification, 133
  clinical types, 146
  course of, 136
  digestion, 146, 401
  experimental, 155
  in malaria, 462
  in newborn infants, 149
  in phthisis, 346
  post-hemorrhagic, 151
  in pregnancy, 148
  relation to immunity, 138
  significance of inflammatory, 133
Leukanemia, 484
Leukemia, 227
  acute, 241, 245, 484
  alkalescence in, 247
  bacteriology, 232
  changes in blood, 239
    leucocytes, 241
    red cells, 240
    in viscera, 233
  Charcot-Leyden crystals in, 248
  chemistry, 247
  diagnosis, 249
  eosinophiles in, 162
  etiology, 230
  extirpation of spleen in, 275
  glycogen in, 248
  history, 227
  intestinal intoxication in, 231
  leucocytosis, antemortem, in, 246
  lymphatic, 244
  lymphocytes in, 243
  malaria as a cause of, 231
  marrow in, 234
  mast-cells in, 244
  nucleins in, 248
  pathogenesis, 237
  peptone in, 248
  protozoa in, 233
  rachitis as a cause of, 231
  relation to pseudoleukemia, 258
  resemblance to a neoplasm, 239
  septicemia in, 246
  specific gravity in, 247
  spermin in, 246
  stomatitis as a cause of, 231
  syphilis as a cause of, 231
  transformation into pernicious anemia, 247
  transformations of type, 247
  tuberculin in, 246
  types of myelocytes in, 241
  tyrosin in, 248
  uric acid in, 248
  variations in blood changes, 245
  xanthin bodies in, 248
Lipacidemia, 83
Lipemia, 83, 374
Liver, 389
  abscess of, 392
  acute yellow atrophy of, 393
  cancer of, 391
  diseases of, eosinophiles in, 162, 393
  echinococcus cyst of, 393
  embryonal, nucleated red cells in, 179
  hypertrophic cirrhosis, 392
Lungs, diseases of, 407
    asphyxia, 408
    bronchitis, 410
    emphysema, 409
    eosinophiles in, 162, 409
Lutein, 79
Lymphatic anemia, 171
  leukemia, transformation into myelemia, 235, 484
    marrow in, 234
Lymphocytes, development of, 184
  large, in pneumonia, 287
  in leukemia, 243
    degenerative changes in, 244
    large, 244
  peculiar, in pneumonia, 288
  proportions, 129
  relation to neutrophile cells, 238
  structure, 123
Lymphocytosis, antemortem, 154
  in cholera infantum, 397
  in congenital syphilis, 171, 342
  in diphtheria, 171, 291
  in epilepsy, 385
  experimental, 172
  large cells in, 172
  in malaria, 462
  occurrence of, 169
  origin of, 169
  in rachitis, 170, 380
  relation to eosinophilia, 172
  in sarcoma, 170, 427
  in typhoid fever, 170, 305
Lymphoma, 255
Lymphosarcoma in pseudoleukemia, 256

MALARIA, 431
    anemia of, 459
      pathogenesis of, 461
  a cause of leukemia, 231
    pernicious anemia, 208
  chronic, 457, 459, 463
  coma in, 456
  conjugation, 454
  crescentic bodies, 450

Malaria, development of parasite in mosquito, 453
  without diminution of red cells, 457
  eosinophiles in, 164, 463
  estivo-autumnal parasite, 443
  extirpation of spleen in, 275
  flagellate bodies, 432, 451
  hemameba immaculata, 444, 449
  hemoglobinemia, 367
  leucocytosis of, 462
  lymphocytosis in, 462
  nucleus and chromatin of parasites, 436, 439, 440, 446
  occurrence of parasites in blood, 455
  without parasites in blood, 457
  phagocytosis in, 463
  pigment, estivo-autumnal, 446
  pigmented leucocytes, 463
  quartan parasite, 442
  quinine, action of, 458
  quotidian parasite, 446
  red cells in, 460
  remittent, 457
  rings, comparison of tertian and estivo-autumnal, 437
    estivo-autumnal signet, 443
    significance of, 436
    tertian, 435
  rosettes, estivo-autumnal, 445
    quartan, 443
    tertian, 441
  species, plurality of, 446
  splenectomy in, 275
  technics, 431
  tertian parasites, 435, 447
  typhoid fever and, 458
Malta fever, 334
Mania, 384
Marrow, extrusion of nuclei of red cells in, 178
  giant cells in, 177
    in chlorosis, 193
  lesions in pernicious anemia, 212
  in leukemia, 234
  in lymphemia, 234
  malarial parasites in, 460
  normal limits of red, 180
Massage, effect in concentrating blood, 19
Mast-cells, development of, 184
  in leukemia, 244
  occurrence of, 167
  proportions, 129
  staining of, 57
  structure, 125
Measles, 300
  German, 301
  protozoa in, 296, 300
Megaloblasts, structure, significance, 99
Megalocytes, occurrence, significance, 98, 361
Melancholia, 384
Melanin, chemistry of, 73
Melena neonatorum, syphilitic, 342
Meningitis, tuberculous, 347
Menstruation, red cells in, 105
Mental diseases, 384
Mercury, effects on blood in syphilis, 339
    on red cells, 113
Methemoglobin, chemistry of, 72
  spectrum of, 30
Methemoglobinemia, 364
Microcytes, occurrence, significance, 97, 219, 361
Miescher's hemoglobinometer, 47
Mosquito, development of malarial parasite in, 453
Mucinemia in myxedema, 381
Muscular exertion, leucocytosis in, 148
Myeloblast, 124
Myelocytes in congenital syphilis, 342
  degenerative changes in, 242
  morphology, 125
  occurrence of, 168
  in pneumonia, 288
  types of, in leukemia, 241
Myxedema, 380
  chemistry of, 381
  mucinemia in, 381
  thyroid therapy in, 381

NAGANA, 480
Nephritis, 415
    chemistry of blood in, 416
    eosinophiles in, 163
Nervous diseases, 384
    eosinophiles in, 163, 385
    influences, effect on quality of blood, 18
  system in chlorosis, 192
    in pernicious anemia, 211
Neurasthenia, 387
Nitrites, poisoning by, 365
Nitrobenzol poisoning, 365
Nocht-Romanowsky method, 434
Normoblasts, occurrence, significance, 98
Nucleated red cells, 98
  transformation into disks, 178
Nuclein, chemistry of, 74
Nucleins in leukemia, 248
Numbers of blood cells, 35

OBESITY, 374
Oligocythemia, 114
Oligoplasmia in cholera, 405
  in chlorosis, 199
Oliver's hemoglobinometer, 48
Operations, surgical, effects on blood, 110
Osmotic relations of blood, 88
  tension, estimation of, 61
Osteomalacia, 377
  chemistry of, 377
  eosinophiles in, 165, 378
Osteomyelitis, 321, 324
Osteosarcoma, 427
Oxyhemoglobin, chemistry of, 72
Oxyuris, 206, 473

PANARITIUM, 324
Parasites, intestinal, 473
  eosinophiles with, 165
  in pernicious anemia, 206
Paratyphoid fever, 307
  bacilli, 314
Paresis, 384
Peptone, in leukemia, 248
  occurrence in blood, 77
Pericarditis, 330
Peritonitis, 330
Pernicious anemia, changes in blood, 216
      chemistry, 216
      Hb, 217
      leucocytes, 221
      red cells, 218
  lesions in marrow, 212
  "mycrocytic," 355, 213
  pathogenesis, 215
  progressive, 191
    etiology of, general, 204
      special, 206
    historical, 202
    scope of term, 205
    relation to pseudoleukemia, 259
    résumé of chief facts, 222
    transformation into leukemia, 231, 484
  serumtherapy in, 362, 484
Perspiration, leucocytosis from, 148
Phagocytosis in malaria, 463
  relation to immunity, 138
Phthisis, leucocytosis in, 346
Plague, 334
Plethora, 17, 106
Pleurisy, 330
Pneumonia, 282
  blood of, bacteria in, 289, 485
  degeneration of leucocytes in, 288
  eosinophilia in, 288
  glycogen in, 288
  hypoleucocytosis in, 286
  lymphocytes, large, in, 287
    peculiar in, 287
  myelocytes in, 288
  toxicity of serum in, 283
Poikilocytosis, 100
Poisoning by antifebrin, 366
  by antipyrin, 366
  by arseniuretted hydrogen, 363
  by carbonic oxide, 366
  by chromic acid, 365
  by guaiacol, 364
  by hydrocyanic acid, 366
  illuminating gas, polycythemia of, 109, 366
  by lead, 113
  by nitrites, 365
  by nitrobenzol, 365
  by phosphorus, polycythemia of, 109
  by potassium chlorate, 363
  by pyrogallol, 366
  by toadstools, 364
Polychromasia, 101, 483
Polycythemia, 106
  after cold baths, 109
  of diarrheal diseases, 109
  of endocarditis, venous stasis, etc., 109, 411
  of high altitudes, 107
  of illuminating gas poisoning, 109, 366
  in jaundice, 391
  of newborn infants, 107
  of phosphorus poisoning, 109
Potassium chlorate poisoning, 364
Precipitin test, 24
Pregnancy, leucocytosis of, 148
  leukemia after, 231
  pernicious anemia after, 207
  red cells in, 106
Protozoa, in leukemia, 233
  in measles, 296, 300
  in variola, 296
Pseudoleukema, 254
  acute, 258
  anatomical lesions of, 255
  changes in blood of, 262
  diagnosis of, 264
  history, 254
  infectious origin of, 260
  lymphosarcoma in, 256
  relation of, to leukemia, 258
    to pernicious anemia, 259
    to tuberculosis, 259
  skin in, 255
  spleen in, 257
Pseudolymphocytes, 125
Psychical emotions, effect on quality of blood, 19
Purges, effect of, on blood, 403
Purpura hemorrhagica, 260, 353
    bacteria in, 354
    non-infectious, idiopathic, 213, 355
Pyemia, 321
Pyrogallol poisoning, 366

QUALITY of blood, effect of nervous influences on, 18
Quantity of blood, 31

RABIES, 333
Rachitis, 378
    as cause of leukemia, 231
    chemistry of, 380
    eosinophilia in, 380
    hyperplasia of spleen in, 380
    lymphocytosis in, 380
Receptors, 142
Red cells, chemical composition, 71
  development of, 175
  fetal, 101
  granular degeneration, 102, 365, 483
  influence of therapeutic measures upon, 111
  morphology and physiology, 96
  numbers of, 104

Red cells, pathological changes in, 100
  size of, 97
  staining reactions, 96
Reduced Hb, chemistry of, 72
"Reizungsformen," Turk's, 244, 288
Relapsing fever, 466
    serum diagnosis in, 468
Resistance of red cells in fever, 278
Respiration, effects on blood, 407
Rheumatism, 327
  bacteria in, 328
  eosinophiles in, 164, 328

SALT solution, infusion of, 364
Sarcoma, 427
    eosinophilia in, 428
    lymphocytosis in, 427
    of spleen, 264
Scarlet fever, 298
    eosinophiles in, 164, 299
    bacteria in, 300
Schistocytosis, 221
Scurvy, 357
  bacteria in, 358
Septicemia, 321
  in leukemia, 246
Serum, albumins of, 75
  chemistry of, 75
  diagnosis of blood, 24
    of leprosy, 350
    of Malta fever, 334
    of plague, 334
    in relapsing fever, 468
    of typhoid fever, 311
  globulicidal action of, 76
  infusion of, 364
  inorganic principles of, 75
  specific gravity of, 76
Serumtherapy in anemia, 362, 484
  in diphtheria, 292
  by hemolysin, 362, 484
  in typhoid fever, 307
  in variola, 297
Sex, influence on numbers of red cells, 105
Skin diseases, eosinophiles in, 163
  lesions in pseudoleukemia, 255
Sleeping-sickness, 485
Smears of blood, method of preparing, 53
Snake poison, 363
Specific gravity, in carcinoma, 426
    estimation of, 57
      Hammerschlag's method, 57
      Schmaltz's method, 58
    general variations of, 84
    in leukemia, 247
    of serum, 76
Spectra of blood pigments, 29
Spectrophotometer, Glan's, 376
Spermin in leukemia, 246
Spindle cells, 186
Spirillum of Obermeier, 466
Spleen, aspiration of, in typhoid fever, 306
  epitheliome primitive of, 264
  extirpation of, in leukemia, 275
    in malaria, 275
  hyperplasia of, in rachitis, 380
  sarcoma of, 264
  syphilis of, 264
Splenectomy, 273
  in animals, 273
  in man, 274
Splenic anemia, 264
Splenocyte, 126, 184
Spotted fever of Montana, 481
Staining methods, 55
    dahlia, for mast-cells, 57
    eosinophiles, methylene blue, 55
    Jenner's, 56
    triacid mixture, 56
    Nocht's, 434
Stippling of red cells, 450
Stomach, carcinoma of, 209, 399, 425
  digestion leucocytosis, 146, 401
  dilatation of, 396
  diseases of, 395
  ulcer of, 398
Stomatitis, a cause of leukemia, 231
Suppuration, 326
Surgical procedures, effects on blood, 110
Surra, 480
Syphilis, 339
  a cause of leukemia, 231
  congenital, 342
    lymphocytosis in, 171, 342
    myelocytes in, 343
  eosinophiles in, 165
  in pernicious anemia, 207
  of spleen, 264

TECHNICS, 23
  in malaria, 431
Tenia, 473
Tests for blood, qualitative, 23
      guaiacum, 24
      serum, 24
      spectroscopic, 28
      Teichmann's hemin, 24
Tetanus, 333
Tetany, 387
Thoma's hematocytometer, 35
Thyroid chlorosis, 387
  therapy in myxedema, 381
Toadstools, poisoning by, 364
Tonsillitis, 329
Toxicity of serum, in pneumonia, 283
Toxins, 143
Transfusion, effects of, 117, 364
Trichinosis, 470
Trypanosomiasis, 480
  in sleeping-sickness, 485
Tuberculin, 171, 246
Tuberculosis, 343
  bacteria in, 348

Tuberculosis of bones, 348
  chemistry of, 348
  meningitis, 347
  relation to pseudoleukemia, 259
Tumors, eosinophiles in blood, 163, 425 428
Typhoid fever, 302,
  aspiration of spleen in, 306
  bacteria in, 306
  bilious, 468
  a cause of pernicious anemia, 208
  cold baths in, 304
  lymphocytosis in, 305
  serum diagnosis of, 311
Typhus fever, 332
Tyrosin in leukemia, 248

UREA, occurrence and estimation of, 80, 417
Uremia, 417
  alkalescence in, 418
  chemistry of, 417
Uric acid, in blood of gout, 326
  in leukemia, 248
  occurrence and estimation of, 80
  "thread test," 377

VACCINIA, 297
  blood in, in children, 297
Varicella, 297
Variola, 294
  protozoa in, 296
Varioloid, 294
Vermiculus, of malaria, 452
Volume of red cells and plasma, 31
  of blood, total, 31

WANDERING cells, primary, 180
  Werlhoff's disease, 353
Whole blood, chemistry of, 77
  quantitative estimation of albumins of, 77
Whooping-cough, 329
Widal's test, 311

XANTHIN bodies in leukemia, 248

YELLOW fever, 331

ZAPPERT chamber, 38

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